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Science observer. Vol. IV. 12, 1886. 8°.

———*American Academy of Arts and Sciences.*
Proceedings. Vol. XX, XXI, 1884–86. 8°.

———*Society of Natural History.*
Memoirs. Vol. III. 12, 13, 1886. 4°.
Proceedings. Vol. XX. 3, XXIII. 1, 2, 1880–86. 8°.

BROOKLYN.—*Entomological Society.*
Entomologica Americana. Vol. I. 3–12, II. 1–3, 1885–86. 8°.
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Bulletin. No. 2, 1886. 8°.

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PAWTUCKET.—*The ornithologist and oölogist.* Vol. X. 7–12, 1885. 8°.
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Bulletin. No. 1, 1885. 8°.
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Bulletin. Vol. I. 1–7, 1884–86. 8°.

UNIVERSITY OF VIRGINIA.—*Leander McCormick Observatory.*
Publications. Vol. I. 1-3, 1885-86. 8°.
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Circulars of information. 1885 i-v, 1886 i. 8°.
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Professional papers. No. 18, 1885. 4°.
———*United States Geological Survey.*
Annual report. III, IV, 1882-83, 1883-84. 8°.
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Proceedings. New series. Vol. III. 4, IV. 1, 2, 1885-86. 8°.

---

ABERDEEN.—*Dun Echt Observatory.*
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Mémoires. Tome VI, 1885-86. 8°.
Bulletin. No. 123-150, 1882-84. 8°.
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———*Kon. Zoologisch Genootschap "Natura Artis Magistra."*
Nederlandsch tijdschrift voor de dierkunde. Jaarg. V. 1, 1884. 8°.
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Bericht. XXVIII, 1885. 8°.
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Bulletin. Tome XXXIX, XL. 1, 1885-86. 8°.
BARCELONA.—*Real Academia de Ciencias Naturales y Artes.*
Acta de la sesion inaugural, 1884-85. 8°.
BASEL.—*Naturforschende Gesellschaft.*
Verhandlungen. Theil VII. 3, 1885. 8°.
BATAVIA.—*Kon. Natuurkundige Vereeniging in Nederlandsch-Indië.*
Natuurkundige tijdschrift. Deel XLIV, XLV, 1885-86. 8°.
Catalogus der bibliotheek. 1884. 8°.
———*Magnetical and Meteorological Observatory.*
Observations. Vol. VI, 1885. 4°.
BERGEN.—*Museum.*
Bidrag til Myzostomernes anatomi og histologi. Af F. Nansen. 1885. 4°.
BERLIN.—*Königl. Sternwarte.*
Berliner astronomisches Jahrbuch. 1870-78, 1888. 8°.
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Journal. No. XLIII, 1885. 8°.

BOMBAY.—*Government Observatory.*
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———*Sassoon Mechanics' Institute.*
Annual report. 1884–85. 8°.
BONN.—*Naturhistorischer Verein der preussischen Rheinlande, Westfalens und des Reg.-Bezirks Osnabrück.*
Verhandlungen. Jahrg. XLII, XLIII. 1, 1885. 8°.
Autoren- und Sachregister zu Bd. I–XL. 8°.
BORDEAUX.—*Académie Nationale des Sciences, Belles-Lettres et Arts.*
Actes. Année XLIV–XLVI, 1882–84. 8°.
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Bericht der meteorologischen Commission. 1883. 8°.
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Jahrbücher. Jahrg. X–XIV, 1880–84. 4°.
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Anales. Tome XIX. 4, XX, XXI, XXII. 1–4, 1885–86. 8°.
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Journal. Vol. LIII. ii. 3, LIV. i, ii, LV. i. 1, 2, ii. 1, 2, 1884–86. 8°.
Proceedings. 1885, 1886, No. 1–7. 8°.
Centenary review of the Asiatic Society of Bengal from 1784 to 1883. 1885. 8°.
———*Geological Survey of India.*
Palæontologia Indica. Ser. II, vol. i. 2 fasc. 5; IV, vol. i. 5; X, vol. iii. 6–8, iv. 1, 2; XIII, vol. i. 4 fasc. 5, 5; XIV, vol. i. 3 fasc. 5, 6; 1885–86. 4°.
Memoirs. Vol. XXI. 3, 4, 1885. 8°.
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CALCUTTA.—*Meteorological Department of the Government of India.*
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CAMBRIDGE.—*Philosophical Society.*
Transactions. Vol. XIII. 3, 1885. 4°.
Proceedings. Vol. V. 4, 5, 1885–86. 8°.

CATANIA.—*Accademia Gioenia di Scienze Naturali.*
Atti. Serie III. Tomo XIX, 1886. 4°.

CHERBOURG.—*Société Nationale des Sciences Naturelles.*
Mémoires. Tome XXIV, 1884. 8°.
Catalogue de la bibliothèque. II. 3, 1883. 8°.

CHRISTIANIA.—*Kong. Norske Universitet.*
Antinoos; eine kunstarchäologische Untersuchung. Von Dr. L. Dietrichson. Universitätsprogramm. 1884. 8°.
Om humanisten og satirikeren Johan Laurenberg. Af Dr. L. Daae. Universitets-program. 1884. 8°.

———*Norwegische Commission der Europäischen Gradmessung.*
Geodätische Arbeiten. Heft IV, 1885. 4°.
Vandstandsobservationer. Heft. III, 1885. 4°.

———*Norwegisches meteorologisches Institut.*
Jahrbuch. 1881–84. 4°.

———*Norwegian North-Atlantic Expedition*, 1876–78.
Publication XIV, XV, 1885–86. 4°.

———*Videnskabs Selskabet.*
Forhandlingar, 1884, 1885. 8°.

CHUR.—*Naturforschende Gesellschaft Graubündens.*
Jahresbericht. Neue Folge. Jahrg. XXVIII, 1883–84. 8°.

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DANZIG.—*Naturforschende Gesellschaft.*
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DORPAT.—*Gelehrte Estnische Gesellschaft.*
Sitzungsberichte. 1885. 8°.

———*Naturforscher-Gesellschaft.*
Archiv für die Naturkunde Liv-Ehst- und Kurlands. Ser. I. Bd. IX. 3. Ser. II. Bd. X. 2. 1885. 8°.
Sitzungsberichte. Bd. VII, 2, 1885. 8°.

DRESDEN.—*Naturwissenschaftliche Gesellschaft Isis.*
Sitzungsberichte und Abhandlungen. 1884, 1885 Jan.-June. 8°.

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Jahresbericht. XXI, 1885. 8°.
Verzeichniss von Forschern in wissenschaftlicher Landes- und Volkskunde Mittel-Europas. Bearbeitet von Paul Emil Richter. 1886. 8°.

DUBLIN.—*Royal Geological Society of Ireland.*
Journal. Vol. XVI. 3, XVII. 1, 1886. 8°.

DUBLIN.—*Royal Irish Academy.*

Transactions. Vol. XXVIII. 17-20, 1884-85. 4°.

Proceedings. Ser. II. Science. Vol. IV. 3, 4. Polite Literature and Antiquities. Vol. II. 6. 1885. 8°.

EDINBURGH.—*Geological Society.*

Transactions. Vol. IV. 3, VI, 1883-85. 8°.

———*Royal Observatory.*

Astronomical observations. Vol XV, 1878-86. 4°.

Micrometrical measures of gaseous spectra under high dispersion. By C. Piazzi Smyth. 1886. 4°.

———*Royal Physical Society.*

Proceedings. Vol. VIII, 1883-85. 8°.

EMDEN.—*Naturforschende Gesellschaft.*

Jahresbericht. LXX, 1884-85. 8°.

FIRENZE.—*Biblioteca Nazionale Centrale.*

Bollettino delle pubblicazioni Italiane ricevute per diritto di stampa. 1886, No. 1-24. 8°.

———*R. Istituto di Studi Superiori Pratici e di Perfezionamento.*

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———*Senckenbergische naturforschende Gesellschaft.*

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Bericht. 1885. 8°.

Reiseerinnerungen aus Algerien. Von Dr. W. Kobelt. 1885. 8°.

GENÈVE.—*Institut National Genevois.*

Bulletin. Tome XXV, XXVI, 1884-85. 8°.

———*Société de Physique et d'Histoire Naturelle.*

Mémoires. Tome XXIX. 1, 1884-85. 4°.

GENOVA.—*Museo Civico di Storia Naturale.*

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GIESSEN.—*Oberhessische Gesellschaft für Natur- und Heilkunde.*

Bericht. XXIV, 1886. 8°.

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———*Philosophical Society.*

Proceedings. Vol. XVI, 1884-85. 8°.

GÖTTINGEN.—*Königl. Gesellschaft der Wissenschaften.*

Nachrichten. 1885. 8°.

GÜSTROW.—*Verein der Freunde der Naturgeschichte in Mecklenburg.*

Archiv. Jahrg. XXXIX, 1885. 8°.

HABANA.—*Real Colegio de Belen.*
Observaciones magneticas y meteorologicas. 1885 i–iii. 4°.
HALLE.—*Kais. Leopoldinisch- Carolinische deutsche Akademie der Naturforscher.*
Leopoldina. Heft XXI, 1885. 4°.
——*Naturforschende Gesellschaft.*
Abhandlungen. Bd. XVI. 3, 1885. 4°.
Bericht. 1884. 4°.
——*Naturwissenschaftlicher Verein für Sachsen und Thüringen.*
Zeitschrift für die gesammten Naturwissenschaften. Bd. LVIII. 2–6, LIX. 1, 2, 1885–86. 8°.
HAMBURG.—*Deutsche Seewarte.*
Archiv. Jahrg. VI, VII, 1883–84. 4°.
Monatliche Uebersicht der Witterung. 1884 Nov., Dez.; 1885. 8°.
——*Wissenschaftliche Anstalten.*
Jahrbuch. Jahrg. II, III, 1885–86. 8°.
HANNOVER.—*Naturhistorische Gesellschaft.*
Jahresbericht. XXXIII, 1883–84. 8°.
HARLEM.—*Musée Teyler.*
Archives. Série II. Vol. II. 2–4, 1885–86. 8°.
Catalogue de la bibliothèque. Livr. 1–4, 1885–86. 8°.
——*Société Hollandaise des Sciences.*
Archives néerlandaises des sciences exactes et naturelles. Tome XX, XXI. 1, 1885–86. 8°.
HELSINGFORS.—*Societas Scientiarum Fennica.*
Acta. Tom. XIV, 1885. 4°.
Öfversigt af förhandlingar. XXVI, 1883–84. 8°.
Bidrag till kännedom af Finlands natur och folk. Häft. XXIXX-XLII, 1884–85. 8°.
——*Societas pro Fauna et Flora Fennica.*
Acta. Vol. II, 1881–85. 8°.
Meddelanden. Häft. XII, XIII, 1885–86. 8°.
Beobachtungen über die periodischen Erscheinungen des Pflanzenlebens in Finland. 1883. 4°.
HOBART.—*Royal Society of Tasmania.*
Catalogue of the library. 1885. 8°.
HONGKONG.—*Observatory.*
Observations and researches. 1885. f°.
JENA.—*Medicinisch-naturwissenschaftliche Gesellschaft.*
Jenaische Zeitschrift für Naturwissenschaft. Bd. XVIII. 4, XIX. 1–4, Supplement 1, 2, 1885–86. 8°.
KIEL.—*Naturwissenschaftlicher Verein für Schleswig-Holstein.*
Schriften. Bd. VI. 2, 1886. 8°.
——*Christian-Albrechts-Universität.*
Dissertationen, etc., 1884–85, (38); 1885–86, (80).
KJÖBENHAVN.—*Kon. Danske Videnskabernes Selskab.*
Oversigt over forhandlinger. 1884 iii, 1885, 1886 i. 8°.
KÖNIGSBERG.—*Königl. physikalisch-ökonomische Gesellschaft.*
Schriften. Jahrg. XXV, XXVI, 1884–85. 4°.
KRAKÓW.—*K. k. Sternwarte.*
Materyaly do klimatografii Galicyi. Rok 1884–85. 8°.
LAUSANNE.—*Société Vaudoise des Sciences Naturelles.*
Bulletin. 2e sér. No. 92, 93, 1885–86. 8°.
LEEDS.—*Yorkshire Geological and Polytechnic Society.*
Proceedings. New series. Vol. IX. 1, 1885. 8°.

LEIDEN.—*Nederlandsche Dierkundige Vereeniging.*
Tijdschrift. Ser. II. Deel I. 1, 2, 1885. 8°.
LEIPZIG.—*Astronomische Gesellschaft.*
Vierteljahrsschrift. Jahrg. XX, XXI. 1, 2, 4, 1885–86. 8°.
Publication. XVIII, 1886. 4°.
———*Kön. sächsische Gesellschaft der Wissenschaften.*
Berichte. Math.-physische Classe. Bd. XXXVI, XXXVII, 1884–85. 8°.
———*Naturforschende Gesellschaft.*
Sitzungsberichte. Jahrg. XI, XII, 1884–85. 8°.
———Zoologischer Anzeiger. No. 198–240, 1885–86. 8°.
LIÉGE.—*Société Géologique de Belgique.*
Annales. Tome X, XII, 1882-83, 1884–85. 8°.
Catalogue des ouvrages de géologie, de minéralogie et de paléontologie ainsi que des cartes géologiques qui se trouvent dans les principales bibliothèques de Belgique. Par G. Dewalque. 1884. 8°.
———*Société Royale des Sciences.*
Mémoires. 2e sér. Tome XI, XII, 1885. 8°.
LISBOA.—*Sociedade de Geographia.*
Boletin. Serie IV. 12, V. 1, 2, 4–12, VI. 1–6, 1884–86. 8°.
Subsidios para a historia do jornalismo nas provincias ultramarinas Portuguezas. Por Brito Aranha. 1885. 8°.
LIVERPOOL.—*Literary and Philosophical Society.*
Proceedings. No. XXXVIII, 1883–84. 8°.
LONDON.—*Geological Society.*
Quarterly journal. Vol. XLI. 3, 4, XLII, 1885–86. 8°.
List. 1886. 8°.
———*Linnean Society.*
Journal. Zoology, no. 103–113; Botany, no. 134–144, 150. 1884–86. 8°.
List. 1884–85, 1885–86. 8°.
Index perfectus ad Caroli Linnæi species plantanum nempe earum primam editionem, collatore F. de Mueller. Melbourne, 1880. 8°.
———*Mathematical Society.*
Proceedings. No. 240–272, 1884–86. 8°.
———*Royal Meteorological Society.*
Quarterly journal. New series. No. 55–60, 1885–86. 8°.
List of fellows. 1885. 8°.
———*Royal Historical Society.*
Transactions. New series. Vol. III. 1, 2, 1885–86. 8°.
———*Royal Microscopical Society.*
Journal. Ser. II. Vol. V. 3–6, VI, 1885–86. 8°.
———*Royal Society.*
Philosophical transactions. Vol. CLXXV, CLXXVI, 1884–85. 4°.
Proceedings. No. 232–247, 1885–86. 8°.
List of council and members. 1884, 1885. 4°.
LUND.—*Universitet.*
Acta. Tom. XIX, XXI, 1882–83. 4°.
Universitets-biblioteks accessions-katalog. 1883, 1885. 8°.
LYON.—*Académie des Sciences, Belles-Lettres et Arts.*
Mémoires. Classe des sciences. Tome XXVIII, 1885. 8°.
Recherches historiques sur les mots plantes males et plantes femelles. Par le Dr. Saint-Lager. Paris, 1884. 8°.
———*Musée Guimet.*
Annales. Tome VIII, IX, 1885–86. 4°.
Revue de l'histoire des religions. Tome XI. 2, 3, XII, XIII, XIV. 1, 1885–86. 8°.

MADRAS.—*Government Observatory.*

Magnetical observations, 1851–55. Madras, 1884. 4°.

Telegraphic determination of difference of longitude. 1884. 4°.

Administrative report of the meteorological reporter for 1884–85. 8°.

Meteorological observations at Singapore, 1841–45. Madras, 1851. 4°.

MADRID.—*Comision del Mapa Geologico de España.*

Boletin. Tomo XII. 1, XIII 1, 1885–86. 8°.

Memorias. Descripcion fisica y geologica de la provincia de Guipuzcoa. Por D. Ramon Adan de Yarza. 1884. 8°.

——*Sociedad Espanola de Historia Natural.*

Anales. Tomo XIV. 3, XV. 1, 2, 1885–86. 8°.

Artropodos del viaje al Pacifico verificado de 1862 a 1865 por una comision de naturalistas enviada por el gobierno Español. Insectos neuropteros y ortopteros, por Ignacio Bolivar. 1884. 4°.

MAGDEBURG.—*Naturwissenschaftlicher Verein.*

Jahresbericht und Abhandlungen. 1885. 8°.

MARBURG.—*Gesellschaft zur Beförderung der gesammten Naturwissenschaften.*

Sitzungsberichte. Jahrg. 1884, 1885. 8°.

METZ.—*Académie.*

Mémoires. 3e sér. Année XII, 1882–83. 8°.

MEXICO.—*Museo Nacional.*

Anales. Tomo III. 7–10, 1885–86. 4°.

——*Ministerio de Fomento.*

Boletin. Seccion meteorologica. Tomo X 43–146, 1885–86. f°.

Estudios de meteorologia comparada. Por Mariano Barcena y Miguel Perez. Tomo I, 1885. 8°.

——*Sociedad Mexicana de Historia Natural.*

La naturaleza. Tomo VII. 5–18, 1885–86. 4°.

MIDDELBURG.—*Zeeuwsch Genootschap der Wetenschappen.*

Archief. Deel VI. 1, 2, 1885–86. 4°.

Naamlijst van directeuren en leden. Verslag van het verhandelde in de algemeene vergadering, 1880–84. 8°.

MILANO.—*Real Istituto Lombardo di Scienze e Lettere.*

Rendiconto. Serie II. Vol. XVII, 1884. 8°.

MODENA.—*Regia Accademia delle Scienze, Lettere ed Arti.*

Memorie. Tomo XX. Serie II. Tomo II. 1885. 4°.

——*Società dei Naturalisti.*

Memorie. Ser. III. Vol. II–IV, 1883–85. 8°.

Rendiconti. Ser. III. Vol. I pp. 105–140, II pp. 1–178, 1883–86. 8°.

MONTPELLIER.—*Académie des Sciences et Lettres.*

Mémoires. Section des lettres. Tome VII. 2, 3, 1884–86. 4°.

———Section des sciences. Tome X. 3, 1883–84. 4°.

———Section de médecine. Tome VI. 1, 1885–86. 4°.

MONTREAL.—*British Association for the Advancement of Science.*

Canadian economics. 1885. 8°.

——*Natural History Society.*

The Canadian record of science. Vol. I. 3, 4, II. 1–4, 1885–86. 8°.

MOSCOU.—*Société Impériale des Naturalistes.*

Nouveaux mémoires. Tome XV. 1–3, 1884–85. 4°.

Bulletin. 1884 ii–iv, 1885. 8°.

Meteorologische Beobachtungen am Observatorium der landwirth. Akademie zu Moskau. Jahrg. 1886 i. 4°.

MÜNCHEN.—*Kön. bayerische Akademie der Wissenschaften.*
Sitzungsberichte. Philosoph.-philolog. und histor. Classe. 1885. 8°.
———Mathemat.-physikal. Classe. 1885. 8°.
J. A. Schmeller. Eine Denkrede von Konrad Hofmann. 1885. 4°.
Sage und Forschung. Festrede von F. Ohlenschlager. 1885. 4°.
Zum Begriff und Wesen der römischen Provinz. Festrede von Alois von Brinz. 1885. 4°.
———*Königliche Sternwarte.*
Annalen. Supplbd. X, XIV, 1871–84. 8°.
MÜNSTER.—*Westfälischer Provincial-Verein für Wissenschaft und Kunst.*
Jahresbericht. XIII, 1884. 8°.
NANCY.—*Académie de Stanislas.*
Mémoires. 5e sér. Tome II, III, 1884–86. 8°.
NAPOLI.—*R. Accademia delle Scienze Fisiche e Matematiche.*
Rediconto. Anno XXII–XXIV, XXV. 1–3, 1883–86. 4°.
———*Zoologische Station.*
Mittheilungen. Bd. VI. 2–4, 1885–86. 8°.
NEWCASTLE-UPON-TYNE.—*North of England Institute of Mining and Mechanical Engineers.*
Transactions. Vol. XXXIV. 4–6, XXXV. 1–4, 1885–86. 8°.
NÜRNBERG.—*Naturhistorische Gesellschaft.*
Jahresbericht. 1885. 8°.
ODESSA.—*Société des Naturalistes de la Nouvelle Russie.*
Zapiski. Tom. IX. 2, X, XI. 1, 1885–86. 8°. Supplément, 1886. 4°.
———Matematicheskoe otdielenie. Tom. I–VI, 1878–85. 8°.
OTTAWA.—*Geological and Natural History Survey of Canada.*
Report of progress, 1882–83–84, with maps. Montreal, 1885. 8°.
Summary report, 1885. 8°.
Contributions to Canadian palæontology. Vol. I. 1. Montreal, 1885. 8°.
———*Royal Society of Canada.*
Proceedings and transactions. Vol. II, III, 1884–85. 4°.
OXFORD.—*Radcliffe Library.*
Catalogue of books added, 1884, 1885. 8°.
PALERMO.—*R. Accademia di Scienze, Lettere e Belle Arti.*
Bollettino. Anno II, III. 1–3, 1885–86. 4°.
PARIS.—*École Polytechnique.*
Journal. Cahier LV, 1885. 4°.
Catalogue de la bibliothèque.
———*Société Nationale d'Acclimatation.*
Bulletin. 4e sér. Tome II. 6–12, III. 1, 3–8, 10, 1885–86. 8°.
———*Société Géologique de France.*
Bulletin. 3e sér. Tome XII. 9, XIII, XIV. 1–7, 1884–86. 8°.
———*Société Mathématique de France.*
Bulletin. Tome IX. 5, X. 3, XI. 3, 5, XIII. 5, 6, XIV. 1–4, 1881–86. 8°.
PISA.—*Società Toscana di Scienze Naturali.*
Memorie. Vol. VI. 2, VII, 1885–86. 8°.
Processi verbali. Vol. IV pp. 231–262, V pp. 1–118, 1885–86. 8°.
POTSDAM.—*Astrophysikalisches Observatorium.*
Publicationen. Bd. IV. 1, V, 1885–86. 4°.
PRAG.—*K. k. Sternwarte.*
Magnetische und meteorologische Beobachtungen. Jahrg. XLV, XLVI, 1884–85. 4°.
Astronomische Beobachtungen, 1884. 4°.

PULKOVA.—*Nicolai-Hauptsternwarte.*
Jahresbericht. 1882–83, 1883–84, 1884–85. 8°.
Tabulæ quantitatum Besselianarum pro annis 1885 ad 1889. Ed. Otto Struve. Petrop., 1885. 8°.
Die Beschlüsse der Washingtoner Meridianconferenz. Von Otto Struve. St. Petersb., 1885. 8°.

REGENSBURG.—*Zoologisch-mineralogischer Verein.*
Correspondenz-Blatt. Jahrg. XXXIX, 1885. 8°.

———*Historischer Verein von Oberpfalz und Regensburg.*
Verhandlungen. Bd. XXXVIII, XXXIX, 1884–85. 8°.

RIGA.—*Naturforscher Verein.*
Correspondenzblatt. Jahrg. XXVII–XXIX, 1884–86. 8°.

RIO DE JANEIRO.—*Instituto Historico, Geographico e Ethnographico do Brasil.*
Revista trimensal. Tomo XLVI–XLVII, 1883–84. 8°.
Catalogo dos manuscriptos, 1884. 8°.
Catalogo das cartas geographicas, hydrographicas, atlas, planos e vistas existentes no bibliotheca. 1885. 8°.

———*Museu Nacional.*
Archivos. Vol. VI, 1885. 4°.
Lettre à M. Ernest Renan à propos de l'inscription phénicienne apocryphe. Par Ladislau Netto. 1885. 8°.
Conférence faite au muséum national le 4 Nov. 1884. Par Ladislau Netto. 1885. 8°.

ROMA.—*Biblioteca Nazionale Centrale Vittorio Emanuele.*
Bollettino delle opere moderne straniere acquistate dalle biblioteche pubbliche governative del regno d'Italia. 1886, No. 1–4. 8°.

———*Reale Accademia dei Lincei.*
Memorie della classe di scienze morali, storiche e filologiche. Ser. III. Vol. VIII, X, XI, XIII, 1883–84. 4°.
Memorie della classe di scienze fisiche, matematiche e naturali. Ser. III. Vol. XIV–XIX. Ser. 4. Vol. II, 1883–85. 4°.
Rendiconti. Vol. I. 13–25, 27, 28, II. i. 1, 4–14; ii. 1–11; 1885–86. 4°.
Annuario. 1886. 16°.

———*Reale Comitato Geologico d'Italia.*
Bollettino. Vol. XV–XVI, 1884–85. 8°.
Relazione sul servizio minerario nel 1882. 8°.

———*Ufficio Centrale di Meteorologia Italiana.*
Annali. Ser. II. Vol. V, 1883. 4°.

ROTTERDAM.—*Bataavsch Genootschap der Proefondervindelijke Wijsbegeerte.*
Nieuwe verhandelingen. 2de reeks. Deel III. 2, 1885. 4°.

ST. GALLEN.—*Naturwissenschaftliche Gesellschaft.*
Bericht. 1883–84. 8°.

ST. PETERSBURG.—*Comité Géologique.*
Mémoires. Tome I. 1–4, II. 1–3, III. 1, 1883–85. 4°.
Bulletins. Tome I–III, IV. 1–7, V. 1–6, 1882–86. 8°.
Bibliothèque géologique de la Russie. I, 1885. 8°.

———*Hortus Petropolitanus.*
Acta. Tom. IX. 2, 1886. 8°.

———*Imp. Russ. Geograf. Obshtchestvo.*
Otchet. God 1885. 8°.

———*Kais. Akademie der Wissenschaften.*
Repertorium der Meteorologie. Bd. IX, 1885. 4°.

———*Physikalisches Centralobservatorium.*
Annalen. Jahrg. 1884. 4°.

*Schweizerische naturforschende Gesellschaft.*
Verhandlungen. Jahresversammlung LXVII, LXVIII, 1884–85. 8°.

STOCKHOLM.—*Entomologisk Forening.*
Entomologisk tidskrift. Arg. VI, 1885. 8°.
———*Kong. Svenska Vetenskaps Akademien.*
Handlingar. Ny följd. Bd. XVIII, XIX, 1880–81. 4°.
Bihang. Bd. VI–VIII, 1881–83. 8°.
Öfversigt. Arg. XXXVIII–XL, 1878–79. 8°.
Meteorologiska iagttagelser. Bd. XX, XXI, 1878–79. 4°.
Lefnadsteckningar. Bd. II. 2, 1883. 8°.
STUTTGART.—*Verein für vaterländische Naturkunde in Württemberg.*
Jahreshefte. Jahrg. XLI, XLII, 1885–86. 8°.
SYDNEY.—*Observatory.*
Results of rain and river observations, 1885. 8°.
———*Royal Society of New South Wales.*
Journal and proceedings. Vol. XVIII, 1884. 8°.
Annual report of the department of mines, New South Wales. 1885. f°.
TACUBAYA.—*Observatorio Astronomico Nacional.*
Anuario. Año XI, 1886. 8°.
Coordenadas geograficas, determinadas por Angel Anguiano. Mexico, 1886. 8°.
THRONDHJEM.—*Kon. Norske Videnskabers Selskab.*
Skrifter. 1884. 8°.
TIFLIS.—*Physicalisches Observatorium.*
Magnetische Beobachtungen. 1879–1883. 8°.
Meteorologische Beobachtungen. 1878–1884. 8°.
Beobachtungen der Temperatur des Erdbodens. 1880–83. 8°.
TOKIO.—*Imperial University of Japan.*
Abhandlungen. No. XII, 1885. 4°.
Calendar. 1886–87. 8°.
———*Seismological Society of Japan.*
Transactions. Vol. VII. 1, IX, 1885–86. 8°.
TORINO.—*Musei di Zoologia ed Anatomia Comparata.*
Bollettino. Vol. I. 1–15, 1886. 8°.
TORONTO.—*Canadian Institute.*
Proceedings. Ser. III. Vol. III. 2, IV. 1, 1885–86.
———*Meteorological Service of the Dominion of Canada.*
Report. 1881. Ottawa, 1883. 8°.
TOULOUSE.—*Académie des Sciences, Inscriptions et Belles-Lettres.*
Mémoires. 8e sér. Tome VI, VII, 1884–85. 8°.
UPSALA.—*Regia Societas Scientiarum.*
Nova acta. Ser. III. Vol. XII. 2, XIII. 1, 1885–86. 4°.
UTRECHT.—*Kon. Nederlandsch Meteorologisch Instituut.*
Nederlandsch meteorologisch jaarboek. 1885. 4°.
———*Provinciaal Utrechtsch Genootschap van Kunsten en Wetenschappen.*
Verslag van het verhandelde in de algemeene vergadering. 1885. 8°.
Aanteekeningen van het verhandelde in de sectie-vergaderingen. 1884–85. 8°.
VENEZIA.—*Istituto Veneto di Scienze, Lettere ed Arti.*
Atti. Ser. VI. Vol. II. 3–10, III. 1–9, 1883–85. 8°.
———Notarisia. Anno I. 1, 2, 1886. 8°.
VICENZA.—*Accademia Olimpica.*
Atti. Vol. XVIII, 1883. 8°.
WIEN.—*Kais. Akademie der Wissenschaften.*
Sitzungsberichte. Mathemat.-naturwiss. Classe. Abth. I. Bd. XC, XCI. 1–4, 1884–85. 8°.
———*K. k. Central-Anstalt für Meteorologie und Erdmagnetismus.*
Jahrbücher. Neue Folge. XX, XXI, 1883–84. 4°.

WIEN.—*K. k. geologische Reichsanstalt.*
Jahrbuch. Bd. XXXV, XXXVI. 1, 1885–86. 8°.
Verhandlungen. Jahrg. 1885, 1886 i–iv. 8°.
WIEN.—*K. k. Naturhistorisches Hofmuseum.*
Annalen. Bd. I. 1, 2, 4, 1886. 8°.
——*K. k. zoologisch-botanische Gesellschaft.*
Verhandlungen. Bd. XXXIV–XXXVI, 1884–86. 8°.
——*Oesterreichische Gesellschaft für Meteorologie.*
Zeitschrift. Bd. XX. 7–12, 1885. 8°.
WIESBADEN.—*Nassauischer Verein für Naturkunde.*
Jahrbücher. Jahrg. XXXIX, 1886. 8°.
WÜRZBURG.—*Physikalisch-medicinische Gesellschaft.*
Sitzungsberichte. Jahrg. 1885. 8°.
ZÜRICH.—*Naturforschende Gesellschaft.*
Vierteljahrsschrift. Jahrg. XXVI–XXIX, 1881–84. 8°.

---

Barus, (Carl) and Strouhal, (Vincent). The electrical and magnetic properties of the iron carburets. Washington, 1885. 8°. *From the Authors.*
Harden, William. A suggestion as to the origin of the plan of Savannah. Savannah. 1885. 8°. *From the Author.*
Hirn, G. A. Notice sur les lois du frottement. Paris, 1884. 4°. *From the Author.*
Klossovsky, A. Les orages au sud de la Russie. Odessa, 1886. 8°.
——Les orages en Russie. Odessa, 1886. 8°. *From the Author.*
Lewis, H. Carvill. Marginal kames. Philadelphia, 1886. 8°.
——A great trap dyke across Southeastern Pennsylvania. Philadelphia, 1885. 8°. *From the Author.*
MacLeod, Jules. La structure des trachées et la circulation péritrachéenne. Bruxelles, 1880. 8°. *From the Author.*
Pickering, Edward C. An investigation in stellar photography conducted at the Harvard College Observatory. Camb. 1886. 4°. *From the Author.*
Russell, H. C. Local variations and vibrations of the earth's surface. . Sydney, 1885. 8°.
——Annual address, Royal Society of New South Wales. Sydney, 1885. 8°. *From the Author.*
Searle, Arthur. The apparent position of the zodiacal light. Boston. 4°. *From the Author.*

# I.—On the Law of Error in Target-Shooting. By E. L. De Forest, Watertown, Conn.

The complete expression for the symmetrical law of error in the position of points in a plane is

$$z=\frac{h_1h_2dxdy}{\pi}e^{-(h_1^2x^2+h_2^2y^2)}, \tag{1}$$

where $z$ denotes the probability that an error committed will fall within any small rectangle $dxdy$ whose coördinates, at its middle point, are $x$ and $y$. The axes should be taken to coincide with the free axes of the group of shot-marks, when these last are regarded as the masses of material points all equal to each other. The origin is at their centre of gravity, and is the point for which the probability $z$ is a maximum. (Compare my article in *The Analyst*, Des Moines, Iowa, vol. viii, p. 73.) Though $dx$ and $dy$ are in strictness infinitesimals, the formula is evidently approximately true when they are regarded as any small finite distances. Points for which $z$ is a given quantity will lie in an ellipse, and all such ellipses are similar and concentric as long as the constants $h_1$ and $h_2$ remain the same. These are determined by the relations

$$h_1=\frac{1}{\rho_1\sqrt{2}}, \qquad h_2=\frac{1}{\rho_2\sqrt{2}}, \tag{2}$$

where $\rho_1$ and $\rho_2$ are the quadratic mean errors in the $x$ and $y$ directions. If the probability of deviation from the maximum is the same in all directions, then

$$\rho^2=\rho_1^2+\rho_2^2=2\rho_1^2=2\rho_2^2 \tag{3}$$

is the squared q. m. error measured directly from the origin, and

$$h=h_1=h_2=\frac{1}{\rho} \tag{4}$$

is the constant to be introduced in (1). Denoting $x^2+y^2$ by $r^2$, (1) is reduced to

$$z=\frac{h^2dx\,dy}{\pi}e^{-h^2r^2}, \tag{5}$$

where the ellipses of equal probability have become circles, and the axes may be taken in any convenient direction. As this formula is

the simplest, it is often adopted in discussing the errors of target-shooting. Even when the form (1) is retained, it is robbed of a portion of its generality by assuming that the axes of X and Y are respectively horizontal and vertical, instead of being coincident with the free axes. Although this assumption seems to have been universally made, it has appeared to me to be of doubtful propriety. The only reasons I have seen stated for its adoption are, that errors caused by the wind are horizontal, while those which depend on the range and the force of gravity are vertical. This takes no account of errors produced by other causes, such as defects or peculiarities in the weapon, imperfect sighting, and fatigue or nervousness in the marksman. These may act obliquely, and so far as we know, are as likely to occur in one direction as another.

As a result of accident, it will happen in general, that the centre of gravity of the shot-marks does not exactly coincide with the true point aimed at, namely, the centre of the target. Accidental deviations from the centre of gravity, or from the free axes drawn through it, are thus of the nature of residual errors, while such deviations from the centre of the target, or from axes drawn through it parallel to the former ones, are of the nature of true errors. In any given case, we can compute the amount of probable deviation of the centre of gravity from the centre of the target. If the actual deviation falls within this amount, or does not much exceed it, we may presume that it is purely accidental, and shifting the position of the computed probability surface (1) so as to make its origin coincide with the centre of the target while its coördinate axes remain parallel to their former positions, we shall have the law of probability of error for future shots. But if the actual deviation is far beyond the probable amount, it indicates the probable existence of some constant causes of error, likely to affect future shots in the same way, and the probability surface must not be shifted, unless we also correct the aim of future shots to correspond with it.

It will in general happen also, as the result of accident, that an actual group of shot-marks will be more elongated in one direction than in the direction at right angles to it, so that one of the squared q. m. errors $\rho_1^2$ $\rho_2^2$ will be greater than the other, even when the probability of error is really the same in all directions. The constants $h_1$ and $h_2$ computed by (2) will thus appear to be different, and the law of error will seem to be as in (1), when it is really of the simpler form (5). But here too, in any given case, we can compute the amount of probable difference between $\rho_1^2$ and $\rho_2^2$, suppos-

ing it to be accidental. Then if the actual difference falls within this amount, or does not much exceed it, we may presume that the probability of error is really the same in all directions, and that formula (5) may be properly used, the axes being taken horizontal and vertical, simply because those directions are most convenient. On the other hand, if the actual difference between $\rho_1^2$ and $\rho_2^2$ is much in excess of its probable value, we must presume that the observed elongation of the group of shot-marks is due to constant causes, likely to have a similar effect on future shots, so that (1) is the most suitable formula to express the law of error, and the axes assumed should be the free axes of the group. It has seemed to me that the question whether formula (1) should be used, and if so, whether the coördinate axes should be made coincident with the free axes, is not a mere matter of opinion, but should be decided by some definite test like the above, applied to an extended set of observations. The most suitable observations for this purpose within my reach are those given by Didion at the close of his *Calcul des Probabilités appliqué au Tir des Projectiles.* Paris, 1858.

His first table gives the positions of 125 shot-marks made by spherical bullets, fired from a rifled pistol at 50 metres, under a charge of one gramme of powder. The weapon was placed on a rest, and aimed at a point 0·430 metres above the centre of the target. The positions of the shot-marks were referred to axes taken horizontally and vertically through that centre. The arith. mean of their ordinates is the ordinate of their centre of gravity, and the arith. mean of their abscissas is its abscissa. We easily find the abscissa $u$ and ordinate $v$ of each shot-mark, referred to axes taken horizontally and vertically through the centre of gravity, and expressed in centimeters. The sums of their squares and of their products are

$$[u^2] = 44211, \qquad [v^2] = 55766, \qquad [uv] = -6655.$$

The angle $\varphi$ which a free axis makes with the U axis is given by

$$\tan 2\varphi = \frac{2\,[uv]}{[u^2]-[v^2]}. \tag{6}$$

Hence log. tan $2\varphi$=·06140, and

$$\varphi = 24^\circ\,31' \qquad \text{or} \qquad \varphi = 114^\circ\,31'. \tag{7}$$

These two values, differing by 90°, represent the inclinations of the two free axes of X and Y to the U axis. Denoting them by $\varphi'$ and $\varphi'+90^\circ$, the coördinates of a shot-mark referred to the free axes will be

$$x = u\cos\varphi' + v\sin\varphi', \qquad y = v\cos\varphi' - u\sin\varphi', \tag{8}$$

and the sums of their squares are

$$\left.\begin{aligned}[x^2] &= [u^2]\cos^2\varphi' + [v^2]\sin^2\varphi' + [uv]\sin 2\varphi',\\ [y^2] &= [v^2]\cos^2\varphi' + [u^2]\sin^2\varphi' - [uv]\sin 2\varphi',\end{aligned}\right\} \tag{9}$$

from which we readily find

$$[x^2] = 41172 \qquad [y^2] = 58806.$$

The squared q. m. errors are therefore

$$\rho_1^2 = \frac{[x^2]}{n-1} = 332{\cdot}03, \qquad \rho_2^2 = \frac{[y^2]}{n-1} = 474{\cdot}24, \tag{10}$$

where $n$ is the number of shots.

Since the coördinates of the centre of gravity are the arith. means of those of the shot-marks, the semi-axes of the ellipse of probable error in the position of the centre of gravity are

$$a = 1{\cdot}1774\frac{\rho_1}{\sqrt{n}} = 1{\cdot}92, \qquad b = 1{\cdot}1774\frac{\rho_2}{\sqrt{n}} = 2{\cdot}29. \tag{11}$$

(*Analyst*, viii, p. 77). In the case we are considering, the horizontal and vertical coördinates of the centre of the target referred to the centre of gravity are

$$u = {\cdot}02, \qquad v = 8{\cdot}25,$$

and when referred to the X and Y axes they are by (8)

$$x = 3{\cdot}44, \qquad y = 7{\cdot}50. \tag{12}$$

These values compared with $a$ and $b$ in (11) show that the actual distance of the centre of gravity from the centre of the target is much greater than it probably would be if it were purely accidental. Hence, to represent the probabilities of error in future shots, the surface (1) should in this instance remain with its vertex at the centre of gravity, and not be shifted to the centre of the target, unless the aim is corrected at the same time.

Having thus determined the most probable position of the origin, we wish next to know whether the actual difference between $\rho_1^2$ and $\rho_2^2$ is much in excess of what might be expected if it were accidental. Its probable amount may be found approximately as follows. When the q. m. error $\varepsilon$ is computed from $n$ observations, the probable error of this determination is known to be

$$\cdot 6745\frac{\varepsilon}{\sqrt{2n}}, \tag{13}$$

and consequently the probable error of $\varepsilon^2$ will be

$$\cdot 6745\varepsilon^2\sqrt{\frac{2}{n}}. \tag{14}$$

Therefore, when $\rho_1^2$ and $\rho_2^2$ are computed as in (10), if the probability of error in the $x$ and $y$ directions is really the same, the probable difference between $\rho_1^2$ and $\rho_2^2$, occurring from accidental causes, will be

$$\Delta^2 = \cdot 6745 \frac{2\varepsilon^2}{\sqrt{n}}, \tag{15}$$

or, if we take approximately $\varepsilon^2 = \frac{1}{2}(\rho_1^2 + \rho_2^2)$,

$$\Delta^2 = \frac{\cdot 6745(\rho_1^2 + \rho_2^2)}{\sqrt{n}}. \tag{16}$$

To apply this to the case in hand, we substitute for $\rho_1^2$ and $\rho_2^2$ their numerical values as in (10), and so get for the probable difference

$$\Delta^2 = 48\cdot 64. \tag{17}$$

The actual value

$$474\cdot 24 - 332\cdot 03 = 142\cdot 21,$$

is so much larger that we are obliged to conclude that in this case it is probably not an accidental but a real difference, likely to affect the distribution of future shots in the same way. Hence (1) is the proper formula to use, and the coördinate axes ought to be taken not horizontally and vertically, but coincident with the free axes of the group of shot-marks.

Didion also gives a table of the positions of the shot-marks made by firing a pistol, apparently similar and with equal charge, 250 times at 100 metres distance, aiming at a point 1·47 m. above the centre of the target. By the same procedure as before, we find

$$[u^2] = 1392560, \qquad [v^2] = 1742890, \qquad [uv] = -301930,$$

where $u$ and $v$ are expressed in centimetres. The inclinations of the free axes to the U axis then are by (6)

$$\varphi' = 29^\circ\, 56', \qquad \varphi' + 90^\circ = 119^\circ\, 56', \tag{18}$$

and with coördinates referring to these axes we have by (9)

$$[x^2] = 1218600, \qquad [y^2] = 1916800,$$

so that the squared q. m. errors are by (10)

$$\rho_1^2 = 4894\cdot 0, \qquad \rho_2^2 = 7698\cdot 0. \tag{19}$$

The semi-axes of the ellipse of probable error in the position of the centre of gravity are by (11)

$$a = 5\cdot 21, \qquad b = 6\cdot 53. \tag{20}$$

But the horizontal and vertical coördinates of the centre of the target are

$$u = 1\cdot 72, \qquad v = 15\cdot 82,$$

which referred to the free axes become

$$x = 9{\cdot}38, \qquad y = 12{\cdot}85. \tag{21}$$

Comparing these with (20), we see that the actual distance of the centre of gravity from the centre of the target is too great to be considered accidental, and we infer as in the former case, that to represent the probabilities of future shots, the vertex of the probability surface should remain at the centre of gravity, and not be changed to the centre of the target, unless the aim is also changed.

The probable difference in this case between $\rho_1^2$ and $\rho_2^2$ is by (16)

$$\Delta^2 = 537{\cdot}2. \tag{22}$$

The actual difference however is

$$7698{\cdot}0 - 4894{\cdot}0 = 2804{\cdot}0,$$

a value so much greater that we are obliged to conclude that it is probably not accidental.

This is what might be expected from the results already obtained with the same kind of weapon at shorter range. If there is really a greater liability to error in one direction than in another, it will naturally show itself at all ranges, with only such differences as might occur by accident. The angles which the direction of greatest error makes with the X axis at the two ranges here considered

$$\varphi' + 90^\circ = 114^\circ\, 31' \quad \text{and} \quad \varphi' + 90^\circ = 119^\circ\, 56',$$

are not very different from each other.

Didion finally gives the coördinates of the trajectories of 100 cannon balls fired, under constant charges and angles of elevation, at distances of 200, 400 and 600 metres. The heights are reckoned, not from the centre of a target, but from the plane of the platform on which the gun-carriage stands. By an easy reduction, we get the coördinates $u$ and $v$ referred to axes taken horizontally and vertically through the centre of gravity, and expressed in metres. For 200 metres range, we find

$$[u^2] = 11{\cdot}82, \qquad [v^2] = 16{\cdot}39, \qquad [uv] = -0{\cdot}18,$$

and (6) gives

$$\varphi' = 2^\circ\, 15', \qquad \varphi' + 90^\circ = 92^\circ\, 15'. \tag{23}$$

Then by (9),

$$[x^2] = 11{\cdot}81, \qquad [y^2] = 16{\cdot}40,$$

and by (10),

$$\rho_1^2 = {\cdot}1193, \qquad \rho_2^2 = {\cdot}1657.$$

The probable difference between $\rho_1^2$ and $\rho_2^2$ is by (16)

$$\Delta^2 = {\cdot}0192. \tag{24}$$

The actual difference is greater, being

$$\cdot 1657 - \cdot 1193 = \cdot 0464.$$

We omit the discussion for the 400 and 600 metre ranges, since the shots here are apparently the same as those used at 200 metres, and the differences in relative position for the same shot at different ranges seem to be largely due to accidental variations in the projec-jectile or the powder. From the three series of trials retained, namely, two of pistol shots and one of cannon shots, it appears that the test requires us to use formula (1), and to make the coördinate axes coincide with the free axes. This therefore, it seems to me, had better be generally done when accuracy is desired, unless indeed our results should hereafter be invalidated by those of other and more extended experiments.

We might also inquire what are the probable values, arising from accidental causes, of the cubes of the c. m. inequalities in $x$ and $y$, when the law of error in either direction is suspected to be unsymmetrical. This question finds an answer in my article on the Unsymmetrical Probability Curve, *Analyst*, vol. x, p. 74. See also *Trans. of the Conn. Academy*, vol. vi, part 1. For determining $a_1$ and $a_2$ the easiest way will perhaps be to compute the values of $[u^3]$, $[v^3]$, $[u^2v]$ and $[uv^2]$. Then from (8) we have

$$\left.\begin{aligned}[x^3]&=[u^3]\cos^3\varphi'+[v^3]\sin^3\varphi' \\ &\qquad +3\sin\varphi'\cos\varphi'([u^2v]\cos\varphi'+[uv^2]\sin\varphi') \\ [y^3]&=[v^3]\cos^3\varphi'-[u^3]\sin^3\varphi' \\ &\qquad -3\sin\varphi'\cos\varphi'([uv^2]\cos\varphi'-[u^2v]\sin\varphi')\end{aligned}\right\} \quad (25)$$

and $a_1$ and $a_2$ are obtained like $a$ in *Analyst*, ix, p. 161. For example, from the first table here tried we get

$$[u^3] = 322100, \qquad [u^2v] = -9600,$$
$$[v^3] = 264700, \qquad [uv^2] = 37400,$$

and consequently by (7) and (29)

$$[x^3] = 269207, \qquad [y^3] = 133301,$$

and the cubed inequalities are

$$\zeta_1^3 = \frac{n\,[x^3]}{n^2-1} = 2153\cdot 8, \qquad \zeta_2^3 = \frac{n[y^3]}{n^2-1} = 1066\cdot 5. \quad (26)$$

From accidental causes alone, they would probably be something like

$$\left.\begin{aligned}(\zeta_1^3) &= \pm\,\cdot 6745\rho_1^3\sqrt{\frac{15}{n}} = \pm\,1413\cdot 7, \\ (\zeta_2^3) &= \pm\,\cdot 6745\rho_2^3\sqrt{\frac{15}{n}} = \pm\,2413\cdot 1.\end{aligned}\right\} \quad (27)$$

The actual value of $\zeta_2^2$ falls far within the probable amount, and that of $\zeta_1^2$ does not so much exceed the probable one as to make us confident that it is anything more than accidental. But if both the inequalities were taken into account, we should have

$$\left.\begin{array}{ll} a_1 = 2\rho_1^2 \div \zeta_1^2 = \cdot 30832 & b_1 = \rho_1^2 = 332\cdot 03 \\ a_2 = 2\rho_2^2 \div \zeta_2^2 = \cdot 88934 & b_2 = \rho_2^2 = 474\cdot 24 \end{array}\right\} \quad (28)$$

and the equation of the surface will be of the same form as in formula (34) of my article in the *Transactions.* The sub-index of the first $b_2$ should fall inside the bracket, instead of outside as there printed.

## II.—Extensions of certain Theorems of Clifford and of Cayley in the Geometry of $n$ Dimensions. By Eliakim Hastings Moore, jr., Denver, Colorado.

### I. General Theorems.

Clifford, at the beginning of his "Classification of Loci" (*Mathematical Papers*, p. 305–331), proves the following theorems:

A. Every proper curve of the $n^{th}$ order is in a flat space of $n$ dimensions or less.

B. A curve of order $n$ in flat space of $k$ dimensions (and no less) may be represented, point for point, on a curve of order $n-k+2$ in a plane, whence

C. A curve of order $n$ in flat space of $n$ dimensions (and no less) is always *unicursal.*

These theorems may be extended. Clifford's nomenclature* and methods of proof are adhered to throughout.

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* For the convenience of the reader who may not have at hand a copy of Clifford's *Mathematical Papers*, the definitions (p. 305–6) are given here.

"By a *curve* we mean a continuous one-dimensional aggregate of any sort of elements, and therefore not merely a curve in the ordinary geometrical sense, but also a singly infinite system of curves, surfaces, complexes, &c., such that one condition is sufficient to determine a finite number of them. The elements may be regarded as determined by $k$ coordinates; and then, if these be connected by $k-1$ equations of any order, the curve is either the whole aggregate of common solutions of these equations, or, when this breaks up into algebraically distinct parts, the curve is one of these parts. It is thus convenient to employ still further the language of geometry, and to speak of such a curve as the complete or partial intersection of $k-1$ loci in flat space of $k$ dimensions, or, as we shall sometimes say, in a $k$-flat. If a certain number, say $h$, of the equations are linear, it is evidently possible by a linear transformation to make these equations equate $h$ of the coordinates to zero; it is then convenient to leave these coordinates out of consideration altogether, and only to regard the remaining $k-h-1$ equations between $k-h$ coordinates. In this case the curve will, therefore, be regarded as a curve in a flat space of $k-h$ dimensions. And, in general, when we speak of a curve as in flat space of $k$ dimensions, we mean that it cannot exist in flat space of $k-1$ dimensions.

* * * By a *surface* we shall mean, in general, a continuous two-dimensional aggregate (which may also be called a *two-spread* or *two-way locus*) of any elements whatever, curves, surfaces, complexes, &c., defined by the whole or a portion of the system of solutions of $k-2$ equations among $k$ coordinates. We shall assume that none of these equations are linear, and then shall speak of the surface as in a flat

THEOREM A. *Every proper* r-*spread of the* $n^{th}$ *order is in a flat space of* $n+r-1$ *dimensions or less.*

For through $n+1$ points of the $r$-spread we can draw an $n$-flat, $R_n$; this meets the $r$-spread $S_r$ in a number of points greater than its order, and, therefore, contains a curve, or 1-spread $S_1$ of the $r$-spread $S_r$.

An $(n+1)$flat $R_{n+1}$ drawn through this $n$-flat $R_n$ and an external point of the $r$-spread $S_r$, for a similar reason, contains a 2-spread $S_2$ of the $r$-spread $S_r$.

Thus, finally, there is reached an $(n+r-1)$flat $R_{n+r-1}$, which completely contains the $r$-spread $S_r$.

An $r$-spread of order $n$, say $S_{r,n,k}$, may lie in a flat space of $k$ dimensions, where $k \leqq n+r-1$; when $k=n+r-1$, the $S_{r,n,n+r-1}$ may be called a *full skew* $r$-spread of order $n$.

THEOREM B. *An* r-*spread of order* n *in a flat space of* k *dimensions (and no less), say* $S_{r,n,k}$, *may be represented, point for point, on an* r-*spread of order* $n-k+r+1$ *in an* $(r+1)$*flat,* $S_{r,n-k+r+1,r+1}$.

Join P, an arbitrary fixed point, to Q, a variable point, both being on the $r$-spread; the resulting $(r+1)$spread $S_{r+1}$ is of the order $n-1$; for a $(k-r)$flat $R_{k-r}$ through P meets the $r$-spread $S_r$ elsewhere in $n-1$ points Q, and, therefore, meets the $S_{r+1}$ in the $n-1$ lines PQ, i. e., in a curve of order $n-1$. Each line PQ meets a fixed $(k-1)$flat $R_{k-1}$ in a point Q′ corresponding to Q; the $(r+1)$spread $S_{r+1}$ meets the fixed flat $R_{k-1}$ in an $r$ spread of order $n-1$. The $r$-spread of the $n^{th}$ order in $k$-flat $S_{r,n,k}$ is thus projected into one of order $n-1$ in a $(k-1)$flat, $S_{r,n-1,k-1}$. A second projection from an arbitrary point upon a fixed $(k-2)$flat $R_{k-2}$ gives an $r$-spread of order $n-2$ in a $(k-2)$flat, $S_{r,n-2,k-2}$.

Thus, finally, after $k-\overline{r+1}$ successive projections the original $S_{r,n,k}$ is represented, point for point, on an $r$-spread of order $n-k+r+1$ in an $(r+1)$flat, $S_{r,n-k+r+1,r+1}$.

But the result may be reached at once. Through any $k-\overline{r+1}$ fixed points P of the $r$-spread and a variable point Q pass a $(k-\overline{r+1})$flat $R_{k-\overline{r+1}}$, cutting a fixed $(r+1)$flat $R_{r+1}$, in a point Q′ corresponding to Q. Thus the $S_{r,n,k}$ is represented, point for point,

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space of $k$ dimensions. We shall in certain cases go further, and speak of an $h$-spread or $h$-way locus, viz: a locus determined by the whole or an algebraically separate portion of the system of solutions of $k-h$ equations among $k$ coordinates; if none of these equations are linear, the $h$-way locus will be said to be in $k$ dimensions."

A *proper* curve or spread is one which does not break up into two or more algebraically distinct parts.

on an $r$-spread in the $(r+1)$flat, $S_{r,(n-k+r+1),r+1}$, the *order* of which is $n-k+r+1$, since a $(k-r)$flat $R_{k-r}$ through the $k-r+1$ fixed points P meets the $r$-spread $S_{r,n,k}$ in $n-k+r+1$ additional points Q which correspond to the $n-k+r+1$ points Q′ in which the line of intersection of the $R_{k-r}$ with the fixed $R_{r+1}$ meets the projected $r$-spread $S_{r,n-k+r+1,r+1}$.

THEOREM C. A quadric $r$-spread is in an $(r+1)$flat, and is *unicursal*, its points having (e. g., by projection from a point on it) a one-one correspondence with those of an $r$-flat $R_r$. Hence, a *full skew* $r$-spread of order $n$, $S_{r,n,n+r-1}$, is *always unicursal*, since, by theorem B, it may be represented on an $r$-spread of order $n-k+r+1=2$ in an $(r+1)$flat. Not only so, but *every flat* section of a full skew $r$-spread is *itself* a full skew spread, and, therefore, *unicursal*. For an $s$-flat $R_s$ cuts a full skew $S_{r,n,k\ (k+1-r+n)}$ in an

$$S_{r+s-k,n,s} \equiv S_{r',n',k'}, \text{ which is } \textit{full skew},$$
$$\text{since} \quad k'+1=r'+n',$$
$$\text{i. e.,} \quad s+1=(r+s-k)+n, \text{ using the given}$$
$$\text{relation,} \quad k+1=r+n.$$

## II. FULL SKEW TWO-SPREADS.

1. *The abbildung-system.*

The full skew two-spread of order $m$ in $R_{m+1}$, $S_{2,m,m+1}$, is unicursal; the curve of intersection with any $m$-flat $R_m$, $S_{1,m,m}$ is also unicursal (I, C); an $R_{m-1}$, the intersection of two $R_m$, and so the axis of a pencil of $R_m$, meets the two-spread in $m$ points ($m$ being the order of the spread); further, in the $R_{m+1}$, the all-including flat, there are $\infty^{m+1}$ $m$-flats $R_m$, i. e., $m+2$ asyzygetic $R_m$. Hence, there is a representation or *Abbildung* of the $S_{2,m,m+1}$, point for point, on a plane $y_1 y_2 y_3$, $\mathfrak{Y}_2$; to an $R_m$-section corresponds a *unicursal* curve, say of order $n$, $\mathfrak{C}^n$; to the $m$ points of intersection of an $R_{m-1}$ correspond $m$ points of intersection of a pencil of curves $\mathfrak{C}^n$; the Abbildung of the $(m+1)$ply infinite system of $R_m$-intersections $S_{1,m,m}$ is the system of curves $\mathfrak{C}^n$, $(m+1)$ply infinite or linearly derivable from $m+2$ asyzygetic curves of the system, all of which are unicursal and have the equivalent of $n^2-m$ common points of intersection, say *base-points* of the system. A Cremona transformation may be found which will change an abbildung-system $\mathfrak{C}^n$ of the spread $S_{2,m,m+1}$ into any other abbildung-system $\mathfrak{C}^{n'}$ of the same spread, since there is a one-one correspondence between the points of the two coincident planes $\mathfrak{Y}_2$ containing the two abbild-

ung-systems. There is, therefore, no loss of generality in assuming as the abbildung-system, the system of curves $\mathfrak{C}^m$ having as base-points an $(m-1)$ple pt. $\mathfrak{O}$ and $m-1$ other points, $\mathfrak{P}_1, \ldots \mathfrak{P}_{m-1}$: the conditions given above are satisfied by this system; for the curves are unicursal; the asyzygetic number is $\overline{2m+1-m-1}=m+2$; the base-points are equivalent to

$$(m-1)^2+(m-1)=m(m-1)=m^2-m=n^2-m$$

common points of intersection.

This abbildung-system may be simplified. Transform the plane $\mathfrak{P}_2$ by a quadric transformation, having $\mathfrak{O}$, $\mathfrak{P}_{m-1}$ and $\mathfrak{P}_{m-2}$, as fundamental points.

A curve $\mathfrak{C}^m$, of order $m$, passing through the two fundamental points $\mathfrak{P}_{m-1}$, $\mathfrak{P}_{m-2}$, and having an $(m-1)$ple pt. at $\mathfrak{O}$, the third fundamental point, and passing through $m-3$ fixed points $\mathfrak{P}_1 \ldots \mathfrak{P}_{m-3}$, is transformed into a curve $\mathfrak{C}'$ of order $m-1=2 \cdot m-(\overline{m-1}+1+1)$, having an $(m-2)$ple pt. at $\mathfrak{O}$, (since the line $\mathfrak{P}_{m-1}\mathfrak{P}_{m-2}$ meets the $\mathfrak{C}^m$ in $m-2$ additional pts.), passing through the $m-3$ fixed points $\mathfrak{P}'_1 \ldots \mathfrak{P}'_{m-3}$ corresponding to the pts. $\mathfrak{P}_1 \ldots \mathfrak{P}_{m-3}$, and not passing through $\mathfrak{P}_{m-1}$ $[\mathfrak{P}_{m-2}]$, (since the line $\mathfrak{O}\mathfrak{P}_{m-2}$ $[\mathfrak{O}\mathfrak{P}_{m-1}]$ does not meet the $\mathfrak{C}^m$ except in $\mathfrak{O}$ and $\mathfrak{P}_{m-2}$ $[\mathfrak{O}$ and $\mathfrak{P}_{m-1}]$).

Thus such a quadric transformation reduces by unity the order of the curves of the abbildung-system, and deletes two of the base-points. By $r$ such quadric transformations, the abbildung-system of curves $\mathfrak{C}^m$, with $\mathfrak{O}$, an $(m-1)$ple point, and $m-1$ other points $\mathfrak{P}$, as base-points, is changed into a system of curves $\mathfrak{C}^{m-r}$ with $\mathfrak{O}$, an $(m-r-1)$ple point, and $m-1-2r$ other points $\mathfrak{P}$, as base-points. The simplest abbildung-systems are, evidently,

(*a*) $m$ even$=2m'$, $r=m'-1$. $\mathfrak{C}^{m'+1}$ with $\mathfrak{O}$ as $m'$ple point and through *one* pt. $\mathfrak{A}$.

(*b*) $m$ odd$=2m''+1$, $r=m''$. $\mathfrak{C}^{m''+1}$ with $\mathfrak{O}$ as $m''$ple point.

2. *The canonical form of the equations of the* $S_{2,m,m+1}$.

Let the $X_s$ $(s=1, \ldots m+3)$ be the homogeneous coordinates in $R_{m+1}$ and the $y_s$ $(s=1, 2, 3)$ be the homogeneous coordinates in $\mathfrak{P}_2$.

(*a*) $m$ even$=2m'$. $\mathfrak{O}$ is $y_1, y_2, y_3=0, 0, 1$; $\mathfrak{A}$ is $y_1, y_2, y_3=1, 0, 0$.

By the simplified abbildung-system set

$$\begin{aligned}
&X_1 : X_2 : X_3 : \ldots\ldots\ldots\ldots \ldots : X_{m'-1} : X_{m'} : X_{m'+1} : \\
&X_{m'+2} : X_{m'+3} : X_{m'+4} : \ldots\ldots\ldots\ldots\ldots : X_{\underset{m+1}{2m'+1}} : X_{\underset{m+2}{2m'+2}} : \\
&y_1^{m'} y_2 : y_1^{m'-1} y_2^2 : y_1^{m'-2} y_2^3 : \ldots\ldots\ldots : y_1^2 y_2^{m'-1} : y_1 y_2^{m'} : y_2^{m'+1} : \\
&y_3 \, [y_1^{m'} : y_1^{m'-1} y_2 : y_1^{m'-2} y_2^2 : \ldots\ldots\ldots\ldots : y_1 y_2^{m'-1} : y_2^{m'}].
\end{aligned}$$

Setting $y_2 = ty_1$ and $y_3 = uy_2$, this becomes

$$X_1 : X_2 : X_3 : \ldots : X_{m'-1} : X_{m'} : X_{m'+1} : X_{m'+2} : X_{m'+3} : \ldots : X_{m+1} : X_{m+2} :: \\ 1 : t : t^2 : \ldots\ldots : t^{m'-2} : t^{m'-1} : t^{m'} : \qquad u\,(1 : t : \ldots\ldots : t^{m'-1} : t^{m'});$$

whence the plexus of equations

$$\left\| \begin{matrix} X_1, X_2, X_3, \ldots\ldots X_{m'-1}, X_{m'}, & X_{m'+2}, X_{m'-3}, X_{m'+4}, \ldots\ldots X_{m+1} \\ X_2, X_3, \ldots\ldots\ldots X_{m'}, X_{m'+1}, & X_{m'+3}, X_{m'+4}, \ldots\ldots X_{m+1}, X_{m+2} \end{matrix} \right\| = 0$$

(*b*) $m$ odd $= 2m'' + 1$. $\qquad$ ⊕ is $y_1, y_2, y_3 = 0, 0, 1$.

In accordance with the simplified abbildung-system, set

$$X_1 : X_2 : X_3 : \ldots\ldots\ldots\ldots\ldots\ldots : X_{m''+1} : X_{m''+2} : \\ X_{m''+3} : X_{m''+4} : X_{m''+5} : \ldots\ldots\ldots\ldots : X_{\substack{2m''+2 \\ m+1}} : X_{\substack{2m''+3 \\ m+2}} :: \\ y_1^{m''+1} y_2^0 : y_1^{m''} y_2^1 : y_1^{m''-1} y_2^2 : \ldots\ldots\ldots\ldots : y_1^1 y_2^{m''} : y_1^0 y_2^{m''+1} : \\ y_3 [y_1^{m''} y_2^0 : y_1^{m''-1} y_2^1 : y_1^{m''-2} y_2^2 : \ldots\ldots : y_1^1 y_2^{m''-1} : y_1^0 y_2^{m''}],$$

or, setting $\qquad y_2 = ty_1$ and $y_3 = uy_1$,

$$X_1 : X_2 : X_3 : \ldots\ldots : X_{m''+1} : X_{m''+2} : \ldots\ldots : X_{m+1} : X_{m+2} :: \\ 1 : t : t^2 : t^3 : \ldots\ldots : t^{m''} : t^{m''+1} : u\,[1 : t : t^2 : \ldots\ldots : t^{m''-1} : t^{m''}];$$

whence the plexus of equations

$$\left\| \begin{matrix} X_1, X_2, X_3, \ldots\ldots X_{m''+1}, & X_{m''+3}, X_{m''+4}, X_{m''+5}, \ldots\ldots X_{m+1} \\ X_2, X_3, \ldots\ldots X_{m''+1}, X_{m''+2}, & X_{m''+4}, X_{m''+5}, \ldots\ldots X_{m+1}, X_{m+2} \end{matrix} \right\| = 0$$

In each case the canonical form exhibits the $S_{2,m,m+1}$ as the locus of the line of intersection of corresponding $m$-flats $R_m$ of $m$ projective pencils of $R_m$; $\qquad X_2 - tX_1 = 0, \quad X_3 - tX_2 = 0$, &c.

In fact, these right lines on and ruling the $S_{2,m,m+1}$ correspond to the right lines on the plane $\mathfrak{R}_2$ through the multiple point ⊕; $y_2 - ty_1 = 0$ is met by a $C^{m'+1}$ or $C^{m''+1}$ (which corresponds to an $R_m$-section of $S_{2,m,m+1}$) in only *one* point.

A full skew *curve* O of order $\begin{matrix} (a)\ m' \\ (b)\ m'' \end{matrix}$ corresponds to the point ⊕. ⊕ is (0, 0, 1); or $u = \infty$, $t$ indeterminate; and, therefore, ⊕ corresponds

(*a*) to $X_1 = X_2 = \ldots\ldots = X_{m'+1} = 0 \qquad \left\| \begin{matrix} X_{m'+2}, X_{m'+3}, \ldots\ldots X_{m+1} \\ X_{m'+3}, \ldots\ldots X_{m+1}, X_{m+2} \end{matrix} \right\| = 0$

(*b*) to $X_1 = X_2 = \ldots\ldots = X_{m''+2} = 0 \qquad \left\| \begin{matrix} X_{m''+3}, X_{m''+5}, \ldots\ldots X_{m+1} \\ X_{m''+4}, \ldots\ldots X_{m+1}, X_{m+2} \end{matrix} \right\| = 0$

or the (unicursal) curve $\begin{matrix} (a)\ m' \\ (b)\ m'' \end{matrix}$ in an $\begin{matrix} m'\text{-} \\ m''\text{-} \end{matrix}$flat. · Cf. Clifford, p. 310.

(*a*) A *line* A corresponds to the *point* ⊗. For ⊗ is (1, 0, 0); or $u$ indeterminate, $t = 0$; and, therefore, ⊗ corresponds to

$$X_2 = X_3 = \ldots\ldots = X_{m'+1} = 0 \qquad X_{m'+3} = X_{m'+4} = \ldots\ldots = X_{m+2} = 0,$$

$m$ $m$-flats $R_m$ in $R_{m+1}$ intersect in $R_1$, a right line.

A *point* corresponds to the *line* $\mathfrak{O}\mathfrak{Z}$, viz: the intersection of the full-skew curve O and the line A. $y_2=0$; or $u=\infty$, $t=0$;

$$X_1=X_2=\ldots\ldots=X_{m+1}=0, \qquad X_{m+2}=X_{m'+4}=\ldots\ldots=X_{m+2}=0.$$

3. *Tangent and osculating flats.*

On the two-spread let Q be a point and C a curve passing through Q; there are at Q a tangent line $R_1$, and osculating 2-flat $R_2$, osculating $R_3$ . . . $R_t$ to the curve C, determined by 2, 3 . . . $(t+1)$ consecutive points (Q included) of the curve; the osculating $t$-flat $R_t$ meets the curve C in $t+1$ points at P. The tangent plane $R_2$ at Q to the two-spread is the locus of tangent lines $R_1$ to all curves C through Q; every $m$-flat $R_m$ through this tangent plane $R_2$ meets the spread in a curve having a double-pt. at Q.

Suppose there were an $s$-flat $R_s$ such that every $R_m$ through it met the two-spread in a curve having a $(t+1)$ple pt. at Q. Take *any* curve C through the point P; *every* $R_m$ through the $R_s$ meets the curve C in $t+1$ points at Q, and, therefore, contains the osculating $R_t$ to C at Q; hence the $R_s$ contains the osculating $t$-flats R to *all* curves C through Q, or say it meets the two-spread at $t$ consecutive points in every direction from Q.

In the case of this *full skew* two-spread it will be shown that at every pt. Q there is an osculating $R_{2t-1}$ containing the osculating $R_t$ to every curve through Q; but probably $s=2t$ for the *general* two-spread in any number of dimensions, $R_k$; $(2t \leqq k$, of course).

*Considerations from the abbildung-system.*

The general abbildung-system (cf. §1) of curves $\mathfrak{C}^m$ has as base-pts. the $(m-1)$ple pt. $\mathfrak{O}$, and the $m-1$ pts. $\mathfrak{P}$; there are $m+2$ asyzygetic curves of the system. Consider those curves of the system which have a $(t+1)$ple pt. at $\mathfrak{Q}$, say $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$; these correspond to the $R_m$-sections of the two-spread which have a $(t+1)$ple pt. at Q. Such a curve $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$ includes the line $\mathfrak{O}\mathfrak{Q}$ $t$ times, and, besides, a supplementary curve $\mathfrak{C}^{m-t}(\mathfrak{Q})$, passing through $\mathfrak{Q}$ and the $m-1$ pts. $\mathfrak{P}$ and having an $(m-t-1)$ple pt. at $\mathfrak{O}$. For say the curve $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$ includes the line $\mathfrak{O}\mathfrak{Q}$ $x$ times, and, therefore, also a supplementary curve $\mathfrak{C}^{m-x}(\mathfrak{Q}^{t+1-x})$ with $(t+1-x)$ple pt. at $\mathfrak{Q}$ and $(m-x-1)$ple point at $\mathfrak{O}$; the line $\mathfrak{O}\mathfrak{Q}$ meets the supplementary curves in $(m-x-1)+(t+1-x)=m+t-2x$ pts. and will be again thrown off, if $m-x<m+t-2x$, i. e., if $x<t$. The asyzygetic number for the system of supplementary curves $\mathfrak{C}^{m-t}(\mathfrak{Q})$ is $(\overline{2m-2t+1}-\overline{m-1}-1=)$ $m-2t+1$. Hence there are $m-2t+1$ asyzygetic $m$-flats $R_m$ meeting

the two-spread in curves with Q as $(t+1)$ple pt.; these $R_m$ in the all-including flat $R_{m+1}$ meet in an $(m+1)-(m-2t+1)=2t$-flat $R_{2t-1}$, which is the osculating $2t$-flat at Q, which includes the osculating $t$-flat $R_t$ at Q to every curve through Q; i. e., which meets the two-spread at $t$ consecutive points in every direction from Q; i. e., which contains the osculating $2(t-1)$flats $K_{2t-2}$ at all points immediately adjacent to Q.

What is the locus of osculating $2t$-flats at points Q along a generator $\tau$ of the two-spread?

If a curve $\mathfrak{C}^m$ degenerates into $\mathfrak{P}\mathfrak{Q}$, say the line $\tau$, taken $t+1$ times and a supplementary curve $\mathfrak{C}^{m-t-1}$ ($\mathfrak{P}$ as $\overline{m-t-2}$ple pt. and through the $m-1$ pts. $\mathfrak{Y}$) the $R_m$ cutting the two-spread in the corresponding section will contain the osculating $2t$-flat at every point Q along the generator $\tau$. The asyzygetic number for the system of supplementary curves $\mathfrak{C}^{m-t-1}$ is $(2m-2t-1)-(m-1)=m-2t$. Hence there are $m-2t$ asyzygetic $m$-flats $R_m$ meeting the two-spread in the generator $\tau$ taken $(t+1)$ times; these in the $R_{m+1}$ meet in an $(m+1)-(m-2t)=(2t+1)$flat $R_{2t+1}$, say the osculating $R_{2t-1}$ along $\tau^{t+1}$ the $(t+1)$ple generator $\tau$, which is the locus of the osculating $R_{2t}$ at the points Q along the generator $\tau$.

*The equations of the osculating flats; the orders of the spreads they generate.*

(*a*) $m$ even $=2m'$. Cf. §2, the simplified abbildung-system; the equations in $t$.

The equations correspond to $m-2t$ asyzygetic curves $\mathfrak{C}^{m'+1}$ including the line $\tau$ $t+1$ times; and to the one other asyzygetic curve of the system including the line $\tau$ $t$ times and passing through the point $Q\equiv\tau v$.

$t=0$. $R_1$, the line $\tau$.

$$\begin{array}{ll} \tau X_1 - X_2 = 0, & \tau X_{m'+2} - X_{m'+3} = 0, \\ \tau X_2 - X_3 = 0, & \tau X_{m'+3} - X_{m'+4} = 0, \\ \vdots & \vdots \\ \tau X_{m'} - X_{m'+1} = 0, & \tau X_{m+1} - X_{m+2} = 0. \end{array}$$

$2m'(=m)$ $R_m$ meeting in $R_1$, the line $\tau$.

The additional $R_m$, $vX_1 - X_{m'+2}=0$, determines the point $\tau v$ on the line $\tau$.

$t=1$. $R_2$, the osculating $R_{2t+1-2}$ along $\tau^{t+1-2}$, the locus of tangent planes of points $v$ along the line $\tau$.

$$\begin{array}{ll} \tau^2X_1 \quad -2\tau X_2 \quad +X_3 \quad =0, & \tau^2X_{m'+2}-2\tau X_{m'+3}+X_{m'+4}=0, \\ \tau^2X_2 \quad -2\tau X_3 \quad +X_4 \quad =0, & \tau^2X_{m'+3}-2\tau X_{m'+4}+X_{m'+5}=0, \\ \vdots & \vdots \\ \tau^2X_{m'-1}-2\tau X_{m'}+X_{m'+1}=0, & \tau^2X_m \quad -2\tau X_{m+1}+X_{m+2}=0. \end{array}$$

$$2(m'-1)=m-2 \; R_m \text{ meeting in } R_2.$$

The additional $R_m$, $v(\tau X_1-X_2)-(\tau X_{m'+2}-X_{m'+3})=0$, determines the tangent plane $R_2$ at the point $\tau v$.

And so, in general, the law of formation is clear.

The osculating $R_{2t+1}$ along $\tau^{t+1}$, the locus of the osculating $R_{2t}$ of points $v$ along the line $\tau$. The equations may be written

$$\begin{array}{ll} X^1(\tau-X^1)^{t+1}=0, & X^{m'+2}(\tau-X^1)^{t+1}=0, \\ X^2(\tau-X^1)^{t+1}=0, & X^{m'+3}(\tau-X^1)^{t+1}=0, \\ \vdots & \vdots \\ X^{m'-t}(\tau-X^1)^{t+1}=0, & X^{m-t+1}(\tau-X^1)^{t+1}=0\,; \end{array}$$

where, after the binomial expansion and multiplication by the external factor, the exponents of the X are to be exchanged for corresponding subscripts; $X^r$ is to be changed to $X_r$.

$$2(m'-t)=m-2t \; R_m \text{ meeting in } R_{2t+1}.$$

The additional $R_m$, $(vX^1-X^{m'+2})\,(\tau-X^1)^t=0$ (the exponents becoming subscripts, as above), determines the osculating $2t$-flat $R_{2t}$ at the point $\tau v$.

The osculating $R_{2t-1}$ along $\tau^t$ lies in the osculating $R_{2t+1}$ along $\tau^{t+1}$; the two $R_m$, $X^1(\tau-X^1)^t=0$, $X^{m'+2}(\tau-X^1)^t=0$, with the $m-2t$ equas. of the $R_{2t+1}$ written above are easily seen to be equivalent to the $m-2(t-1)$ equations of the $R_{2t-1}$,

$$\begin{array}{ll} X^1(\tau-X^1)^t=0 & X^{m'+2}(\tau-X^1)^t=0 \\ \vdots & \vdots \\ X^{m'-t+1}(\tau-X^1)^t=0 & X^{m-t+2}(\tau-X^1)^t=0. \end{array}$$

Thus the equations show that the singly infinite system of osculating $2t$-flats $R_{2t}$ at points $v$ along the generator $\tau$ lie in the osculating $(2t+1)$flat $R_{2t+1}$ along $\tau^{t+1}$; and, at the same time, form a *pencil* of $R_{2t}$ in the $R_{2t+1}$ having as an *axis* the osculating $(2t-1)$ flat $R_{2t-1}$ along $\tau^t$; and the osculating flats of the pencil are *homographic** with their points of osculation along the generator $\tau$.

Compare the well known theorem: Salmon; *Geometry of Three*

* The only equation introducing $v$ is *linear* in $v$.

*Dimensions*, § 459; "the anharmonic ratio of four tangent planes passing through a generator of a ruled surface is equal to that of their four points of contact."

(*b*) $m$ odd $= 2m'+1$.

There is one more equation in each case from the first group of coordinates, $X_1 \ldots X_{m'+2}$, than from the second group, $X_{m'+3} \ldots X_{m+2}$; the $v$ equation is of exactly the same nature; the conclusions stated above hold equally for $m$ odd or even.

As the line $\tau$ generates the two-spread $S_{2,m,m+1}$ the osculating $R_{2t+1}$ along $\tau^{t+1}$ generates a $(2t+2)$spread of order $(t+1)(m-2t)$ $S_{2t+2,(t+1)(m-2t),m+1}$.

Let the $m-2t$ asyzygetic $R_m$ determining $R_{2t+1}$ in terms of $\tau$ be $A_m, A_{m-1}, \ldots A_{2t+1}$; the A involve $\tau$ to the power $t+1$; let $A_{m,s}, A_{m-1,s}, \ldots A_{2t+1,s}$, be what the A become when the coordinates of a point $P_s$ in the $(m+1)$flat are substituted for the current coordinates. The $(2t+2)$spread is met by an $(m-2t-1)$flat in say $x$ points $P_s$; any point in the $(m-2t-1)$flat may be given in terms of $m-2t$ asyzygetic points $P_m \ldots P_{2t+1}$;

$$\text{say } P_s = \lambda_m P_m + \lambda_{m-1} P_{m-1} + \ldots + \lambda_{2t+1} P_{2t+1},$$
$$\text{i. e., } X_{1,s} = \lambda_m X_{1,m} + \lambda_{m-1} X_{1,m-1} + \ldots + \lambda_{2t+1} X_{1,2t+1}, \text{ etc.}$$

Substituting the coordinates of $P_s$ in the $m-2t$ $A_r$, and eliminating from the $m-2t$ $A_{r,s}$ the $m-2t$ $\lambda$ which enter homogeneously in *the first degree*, we have the determinant of the order $m-2t$

$$\begin{vmatrix} A_{m,m}, & A_{m,m-1}, & \ldots & A_{m,2t+1} \\ A_{m-1,m}, & A_{m-1,m-1}, & \ldots & A_{m+1,2t+1} \\ \cdot & & & \\ \cdot & & & \\ \cdot & & & \\ A_{2t+1,m}, & A_{2t+1,m-1}, & \ldots & A_{2t+1,2t+1} \end{vmatrix} = 0,$$

an equation of degree $(t+1)(m-2t)$ in $\tau$; for each value of $\tau$, there is one set of values of the $\lambda$, one point $P_s$. Hence the order of the $(2t+2)$spread, the locus of osculating $R_{2t+1}$, is $(t+1)(m-2t)$, as stated. Through a point $P_s$ an osculating $R_{2t+1}$ along $\tau^{t+1}$ may be drawn; this contains the osculating $R_{2t-1}$ along $\tau^t$; the $R_{2t}$ joining the point $P_s$ with this $R_{2t-1}$ is the osculating $R_{2t}$ at some point $v$ of the line $\tau$. This may be expressed thus; through an $R_{m-2t-1}$ may be passed $(t+1)(m-2t)$ $m$-flats $R_m$ which meet the spread in a $(t+1)$ple line $\tau$; and $(t+1)(m-2t)$ $(m-1)$flats $R_{m-1}$ which meet the spread in a $(t+1)$ple point $\tau v$.

(a) $m$ even $= 2m'$. The simplified abbildung-system; § 1.

$\mathfrak{C}^{m'+1}$ may degenerate into the line $\tau$ taken $m'$ times, and a line through $\mathfrak{A}$; for this the limiting case $t=m'-1$.

The $(2t+2)$spread, locus of osculating $R_{2t+1}$ along $\tau^{t+1}$ is of order $(t+1)(m-2t)$. But the $(m-2t)$spread, locus of osculating $R_{m-2t-1}$ along $\tau^{m'-t}$ is also of order

$$(m'-t)(m-2\overline{m'-t-1})=(t+1)(m-2t).$$

For instance, $t=0$; the two-spread locus of lines $R_1$ (i. e., the original two-spread) is of order $m$; and, also, the $m$-spread locus of osculating $R_{m-1}$ along $\tau^{m'}$ is of order $m$.

Thus, when $m$ is even, the orders of the $(2t+2)$spread for $t=t_1$, $t=t_2$, are equal, if $t_1+t_2=m'-1$.

(b) $m$ odd $= 2m''+1$.

$t \lesseqgtr m$; since the curve $\mathfrak{C}^{m''+1}$ in the limiting case degenerates into the line $\tau$ taken $t+1=m''+1$ times.

For $t=m''$ the order of the $(2t+2)=2m''+2=(m+1)$spread is $(m''+1)(m-2m'')=m''+1$; i. e., through *every point* $R_{m-2t-1=0}$ of $R_{m+1}$ may be passed $m''+1$ $R_m$ meeting the 2-spread in an $(m''+1)$ple line $\tau$.

There is no symmetry analogous to that for $m$ even.

4. *Curves on the two-spread.*

(b) $m$ odd $= 2m''+1$.

The abbildung-system, $\mathfrak{C}^{m''+1}$ having $\mathfrak{B}$ as $m''$-ple pt.

Let us denote the *unique* curve of order $m''$ in an $m''$-flat corresponding to $\mathfrak{B}$ by O; and the right lines of the spread by $\tau$. A curve on the spread of order $p$, meeting the unique curve O in $q$ points and every line $\tau$ in $r$ points may be written $C^p(O^q\tau^r)$.

A curve on the abbildung plane $\mathfrak{C}^s(\mathfrak{B}^t)$ of the order $s$ with a $t$-ple point at $\mathfrak{B}$ is met by a curve of the system $\mathfrak{C}^{m''+1}$ in $s(m''+1)-tm''$ points and by any line $\tau$ in $s-t$ points; therefore, it corresponds to a curve on the spread of order $s(m''+1)-tm''$, which meets the unique curve O in $t$ points, and every line $\tau$ in $(s-t)$ points, say

$$C^{s(m''+1)-tm''}(O^t\tau^{s-t}).$$

So a curve $\mathfrak{C}^s(\mathfrak{B}^t\tau^{s-t})$ transforms into $C^{s(m''+1)-tm''}(O^t\tau^{s-t})$.

A curve $C^p(O^q)$, of order $p$, meeting the curve O in $q$ pts., must have

$$p>q \text{ and } p\equiv q \ (\text{mod. } \overline{m''+1}); \text{ say } \begin{array}{l} p=s(m''+1)-tm'', \\ q=t. \end{array}$$

$s(m''+1)-tm''=p. \quad (s \geqq t).$

$s=1, t=1, p=1.$ A line $\tau$ on $\mathfrak{A}_2$ corresponds to a line $\tau$ on the spread.

$s=s, t=s, p=s.$ $s$ lines $\tau$ correspond to $s$ lines $\tau$.

If $s>t$, $p \geqq m''+1$; which shows that the curve O of order $m''$ *is unique*, since it is the only curve of so low an order on the two-spread (the lines $\tau$ excepted).

Curves on the two-spread and in flats of less than $m+1=2m''+2$ dimensions are *full skew* curves. Such a curve is an $R_m$-intersection or a part of an $R_m$-intersection; therefore, its abbildung is a curve $\mathfrak{C}'(\mathfrak{O}^{p-1})$; any $m''+1-s$ lines $\tau$ belong to the supplementary system, of which the asyzygetic number is $m''+2-s$; therefore, $m''+2-s$ asyzygetic $R_m$ meet in an $R_{m''+s}$ in which the curve $C^{s(m''+1)-(s-1)m''-m''+}$ lies. A curve $C^{m''+s}$ in $R_{m''+s}$ is a full skew curve. (I; theorem C.)

The general plane curve $\mathfrak{C}'$ ($t=0$; not through $\mathfrak{O}$) corresponds to a $C^{s(m''+1)}$ which does not meet the unique curve. The *plane* is a full skew 2-spread $S_{2,1,2}$ ($m=2m''+1=1$, $m''=0$). All the geometry of *plane* curves depending upon intersections and tangencies and the order of curves is immediately applicable to the *general* full skew two-spread of *odd* order $m=2m''+1$, the curves $C^{s(m''+1)}$ on the two-spread corresponding completely to the curves $\mathfrak{C}'$ of the plane. A curve $C^{s(m''+1)-tm''}(O^t)$ meeting the unique curve O $t$ times is a particular case of $C^{s(m''+1)}$, and in fact plays the same rôle as a $C^{s(m''+1)}$ having a $t$-ple point. A few examples are given.

There is a double infinity of curves $C^{m''+1}$; two meet in one point; one is determined by two points; they correspond to the lines $\mathfrak{C}^1$ of the plane. A line $\tau$ together with the unique curve O is a special case of a curve $C^{m''+1}$. Five points determine a curve $C^{2(m''+1)}$; which corresponds to a conic $\mathfrak{C}^2$ of the plane.

Pascal's theorem becomes:

If six points $P^1 \ldots P^6$ lie on a curve $C^{2(m''+1)}$ the three points of intersection of the two curves $C^{m''+1}$ joining $P^1P^2$, $P^4P^5$; $P^2P^3$, $P^5P^6$; $P^6P^1$, $P^3P^4$, respectively, lie on another curve $C^{m''+1}$.

To a curve $C^{s(m''+1)-tm''}(O^t\tau^{s-t})$ there are $s(s-1)-t(t+1)=(s+t)(s-t-1)$ tangent lines $\tau$, and $s(s-1)-t(t-1)=(s-t)(s+t-1)$ tangent curves $C^{m''+1}$ in a pencil through a point P.*

An $m$-spread of order $P$ meets the 2-spread in a curve $C^{mP}$ meeting the unique curve O $m''P$ times, and every line $\tau$ in $P$ points; $\therefore C^{mP} \equiv C^{(2m''+1)P}(O^{m''P}\tau^P)$. $s=(m''+1)P$, $t=m''P$. To an $m$-spread of order $P$ in $R_{2m''+2-m+1}$ there are $mP(P-1)$ tangent lines lying entirely on the $S_{2,m,m+1}$.

Two curves $C(O^t\tau^{s-t})$, $C(O^{t'}\tau^{s'-t'})$, meet in $ss'-tt'$ points; in particular, two curves $C(O^t\tau^{s-t})$ meet in $s^2-t^2$ points; one is determined by

* These formulæ are similar to some given by Chasles, *Comptes Rendus*, 1861; cf. the following (a).

$\frac{1}{2}\{s(s+3)-t(t+1)\}$ points. Hence two curves $C(O^t\tau^{s-t})$ through $\frac{1}{2}\{s(s+3)-t(t+1)\}-1$ points determine a pencil of such curves through these and $\frac{1}{2}\{s(s-3)-t(t-1)\}+1$ additional points.

(a) $m$ even $=2m'$. The simplified abbildung-system, $\mathfrak{C}^{m'+1}$ having $\mathfrak{P}$ as $m'$-ple point and $\mathfrak{Q}$ as an ordinary point.

Curves $\mathfrak{C}^{p+1}(\mathfrak{P}_p\mathfrak{Q}^1)$ of order $p+1$ having $\mathfrak{P}$ as $p$-ple pt., through $\mathfrak{Q}$, correspond to *full skew* curves of order $m'+p$; these are the only curves on the spread in a flat of less than $m+1$ dimensions. The proof is like that of (b) for $m$ odd.

In particular:—A line $y_2-\tau y_1=0$ through $\mathfrak{P}$ corresponds to a line $\tau$ on the spread (§ 2); two lines $\tau$ do not meet. A line $v$ through $\mathfrak{Q}$ corresponds to a full skew curve $v$ of order $m'$ on the spread; two curves $v$ do not meet. Through every point on the spread pass one line $\tau$ and one curve $C^{m'}$, $v$; a line $\tau$ and a curve $v$ meet in one point.

$\mathfrak{P}$ corresponds to a curve O of order $m'$, meeting every line $\tau$.

$\mathfrak{Q}$ corresponds to a line A of order $m'$, meeting every curve $v$.

The curve O meets the line A in a point OA. (§ 2.)

In fact, the curve O, the line A, the point OA in no way differ from an ordinary curve $v$, line $\tau$, point $\tau v$ of the spread.

Observe that a curve $\mathfrak{C}^s(\mathfrak{P}^t\mathfrak{Q}^u)$ of order $s$ (having O a $t$-ple and A an $u$-ple point), corresponds to a curve $C^{(s-t)m'+(s-u)}(\tau^{s-t}v^{s-u})$ of order $(s-t)m'+(s-u)$ meeting every line $\tau$ in $s-t$ points and every curve $v$ in $s-u$ points: it passes $s-t-u$ times through the point OA and meets the line A elsewhere in $u$ points, the curve O elsewhere in $t$ points; ($\therefore$ in all, it meets the line A in $s-t$ points, the curve O in $s-u$ points).

A curve $\mathfrak{C}^{s'=(s-t)+(s-u)}(\mathfrak{P}^{t'=(s-u)}\mathfrak{Q}^{u'=(s-t)})$ with an $(s-t-u)$ple point at say $\mathfrak{Q}$ corresponds to a curve $C^{(s-t)m'+(s-u)}(\tau^{s-t}v^{s-u})$ with an $(s-t-u)$-ple point at Q. Thus the order and character of intersection with the lines $\tau$ and curves $v$ and the $(s-t-u)$ple point are exactly the same. The asyzygetic numbers are equal; as shown by the following equality (where $v=s-t-u$, $s'=s+v=2s-t-u$, $t'=s-u$, $u'=s-t$),

$$\begin{aligned}&(s+1)(s+2)-t\underset{\mathfrak{P}^t}{(t+1)}-u\underset{\mathfrak{Q}^u}{(u+1)}\\ =&(s'+1)(s'+2)-t'\underset{\mathfrak{P}^{t'}}{(t'+1)}-u'\underset{\mathfrak{Q}^{u'}}{(u'+1)}-v\underset{\mathfrak{Q}^v}{(v+1)}\end{aligned}$$

The statement above is justified, and it is therefore proper to consider only curves which have *no* especial relation to the point OA; i. e., in the abbildung-plane, only curves $\mathfrak{C}^s(\mathfrak{P}^t\mathfrak{Q}^u)$ where $s=t+u$.

The spread is *ruled* with the lines $\tau$ (§2); and *also* with the curves $v$, $C^{m'}$. The curves $v$ correspond to $y_2-vy_2=0$; the $(m'+2)$spread

$$\left\|\begin{matrix} X_{m'+2}, & X_{m'+3}, & X_{m'+4} & \ldots\ldots & X_{m+1} \\ X_{m'+3}, & X_{m'+4} & \ldots\ldots & X_{m+1}, & X_{m+2} \end{matrix}\right\| = 0$$

is cut in the curves $v$ by $m'$-flats, the intersection of corresponding $R_m$ in the $m'+1$ projective pencils of $m$-flats $R_m$,

$$X_{m'+2} - vX_1 = 0, \quad X_{m'+3} - vX_2 = 0 \ \ldots\ldots \ X_{m+2} - vX_{m'+1} = 0.$$

A curve $\mathfrak{C}^{t+u}$ ($\mathfrak{G}^t\mathfrak{J}^u$) corresponds to a curve $C^{um'+t}(\tau^u v^t)$ of order $um'+t$ meeting each line $\tau$ in $u$ points, each curve $v$ in $t$ points.

Since the number of intersections of curves on the two-spread with the lines $\tau$, the curves $v$ and with each other, and all intersection- and tangency-properties, depend only on the abbildung-curves, it is clear that there is a *complete correspondence* between the curves on a two-spread of even order $m=2m'$, and those on an ordinary hyperboloid or quadric ($m'=1$); the two systems of ruling curves, the lines $\tau$ and the curves $v$ of order $m'$, answer to the two systems of generators on the quadric (only, in the latter case, the two systems being of the same order are indistinguishable). Hence many of Chasles' results (*Comptes Rendus*, liii, 1861) concerning "Propriétés générales des courbes gauches tracées sur l'hyperboloide" apply in this more general case; for example:

A curve $C^{um'+t}(\tau^u v^t)$ is determined by $tu+(t+u)$ points.

Two curves $C^{um'+t}(\tau^u v^t)$, $C^{u'm'+t'}(\tau^{u'} v^{t'})$, meet in $tu'+ut'$ points.

All curves $C^{um'+t}(\tau^u v^t)$ going through $tu+(t+u)-1$ fixed points form a pencil passing through $tu-(t+u)+1$ other fixed points; since any two meet in $2tu$ points.

To a curve $C^{um'+t}(\tau^u v^t)$ $2t(u-1)$ lines $\tau$ are tangent.
$2u(t-1)$ curves $v$ are tangent.
$2tu$ curves $C^{m'+1}(\tau^t v^t)$ of a pencil through a pt. P are tangents.

These numbers are easily derived by considering the corresponding abbildung-curves.

The curve $C^{um'+t}(\tau^u v^t)$ corresponds to a curve $C^{u+t}(\tau^u v^t)$ on an ordinary quadric; on the quadric there is no distinction between the curves $C^{u+t}(\tau^u v^t)$, $C^{u+t}(\tau^t v^u)$; i. e., two curves of the same order $t+u$, which meet the generators of one system $\tau$ in $\substack{u\\t}$ points, and those of the other system $v$ in $\substack{t\\u}$ points. If, then, there is a theorem about curves of order $u_r m'+t_r$ ($r=1, 2, \ldots S'$) meeting the lines $\tau$ in $u_r$ pts. and the curves $v$ in $t_r$ pts., the *same* theorem will be true about corresponding curves of order $t_r m'+u_r$ meeting the lines $\tau$ in $t_r$ pts. and the curves $v$ in $u_r$ pts.

A curve $C^P(\tau^u v^t)$ must have $P=um'+t$.

An $m$-spread of order $P$ intersects the 2-spread in a curve of order $mP=2m'P$, meeting the lines $\tau$ in $u=P$ points, and the curves $v$ in $t=m'P$ points; say $C^{2m'P}(\tau^P v^{m'P}) \equiv C^{Pm'+m'P}(\tau^P v^{m'P})$.

$$2t(u-1)=2m'P(P-1)=mP(P-1)$$

lines $\tau$ are tangent to this curve.

There are $mP(P-1)$ lines $\tau$ lying entirely on the two-spread $S_{2,m,m+1}$ and tangent to an $m$-spread $S_{m,P,m+1}$ of order $P$.* (m *odd* or *even*; cf. § 4, b).

The curves on a full skew two-spread of even order, $m=2m'$, then, have an exact correspondence with those on an ordinary quadric, $m=2$; those on a full skew two-spread of odd order, $m=2m''+1$, have almost as exact a correspondence with the curves on a plane, $m=1$; the *unique* curve O is a singularity, but curves meeting it in $t$ points play very much the same rôle on the two-spread as curves having a $t$-ple pt. at an ordinary point of the spread. These close correspondences with the hyperboloid and plane curves are the marked features of the theory of curves on full skew two-spreads.

## III. Spreads of Odd Orders on Quadrics.

The known theorems, that a curve $S_1$ of odd order on an ordinary quadricone, cone-$Q_{2,3}$, passes an odd number of times through the vertex V, and that a general quadric 3-spread $Q_{3,4}$ contains no 2-spreads $S_2$ of odd order (cf. Clifford, *Mathematical Papers*, p. 644), may be extended.

$Q_{r,r+1}$ will denote a *general* quadric $r$-spread in $R_{r+1}$, and cone-$Q_{r,r+1}$ a quadric $r$-spread in $R_{r+1}$ formed by joining a (general) $Q_{r-1,r}$ to a vertex-point V in $R_{r+1}$.

The section of $Q_{r,r+1}$ made by a tangent $r$-flat $R_r$ is a cone-$Q_{r-1,r}$.

The section of a cone-$Q_{r,r+1}$ by an $R_r$ through the vertex V is a cone-$Q_{r-1,r}$; but that by an arbitrary $R_r$ is a (general) $Q_{r-1,r}$.

$S_r$ will denote an $r$-spread.

$A_1$. The general $Q_{2m,2m+1}$ has on it no $r$-spread of odd order unless $r<m+1$.

$A_2$. The cone-$Q_{2m,2m+1}$ has on it no $r$-spread of odd order

$A_{21}$. unless $r<m+1$; while

$A_{22}$. $m$-spreads of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order pass an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times through the vertex V.

$B_1$. The general $Q_{2m-1,2m}$ has on it no $r$-spread of odd order unless $r<m$.

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* Chasles gives this for the case $m=2$.

$B_1$. The cone-$Q_{2m-1,\,2m}$ has on it no $r$-spread of odd order unless

$B_{21}$. $r < m+1$; while

$B_{22}$. $m$-spreads of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$order pass an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$number of times through the vertex V.

Let $B_1$ hold in $R_{2n}$; then will the theorems A hold in $R_{2n+1}$.

$A_1$. $A_{21}$. Take arbitrary $R_{2n}$ section of $\left.\begin{matrix}Q_{2n,\,2n+1}\\ \text{cone-}Q_{2n,\,2n+1}\end{matrix}\right\}$ containing an $r$-spread of odd order; there results a (general) $\left.\begin{matrix}Q_{2n-1,\,2n}\\ Q_{2n-1,\,2n}\end{matrix}\right\}$ with an $(r-1)$spread of odd order on it; hence, by $B_1$, $r-1<n$; or $r<n+1$; proving $A_1$, $A_{21}$.

$A_{22}$. Notice that the projection in $R_{2m+1}$ from a pt. P upon an $R_{2m}$ of an $r$-spread of order $s$ passing $t$ times through P is an $r$-spread of order $\overline{s-t}$, i. e., of *odd* order, unless $s-t$ is even; i. e., unless the $r$-spread of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order passes an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times through P.

Take arbitrary $R_{2n}$ section of cone-$Q_{2n,\,2n+1}$ containing an $n$-spread $S_n$; it is a $Q_{2n-1,\,2n}$ which contains the projection through P of $S_n$; if $B_1$ holds, the preceding consideration shows that $A_{22}$ must also.

Hence, if the propositions hold for $R_{2n}$ (i. e., the B), they do for $R_{2n+1}$ (i. e. the A).

If the A ($m_A=n$) hold for $R_{2n+1}$, the B ($m_B=n+1$) will hold for $R_{2n+2}$.

$B_1$. The $Q_{2n+1,\,2n+2}$ contains an $r$-spread $S_r$ of odd order; the tangent $R_{2n+1}$ at a pt. V (not on the $r$-spread $S_r$), cuts the quadric in a cone-$Q_{2n,\,2n+1}$, containing an $(r-1)$spread of odd order not passing through the vertex V; hence, by $A_{21}$,

$$r-1<n,\ r<n+1,\ r<m_B,\ \text{proving } B_1.$$

$B_2$. An $R_{2n+1}$-section through the vertex V of the cone-$Q_{2n+1,\,2n+2}$ shows the dependence of $B_{21}$, $B_{22}$ on the truth of $A_{21}$, $A_{22}$.

But $A_2$ holds for $R_3$. A curve of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order in an ordinary quadricone, cone-$Q_{2,\,3}$, passes through the vertex V an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times, because its projection through V upon a plane $R_2$ is (a conic) of *even* order.

From $A_2$ for $R_3$ follow at once the B for $R_4$, and thus the general propositions as enunciated for $R_{2m}$, $R_{2m+1}$.

## IV. Flats on Quadrics.

Prof. Cayley, "On the Superlines of a Quadric Surface in 5-dimensional space" (*Quart. J. M.*, 1872–3, t. xii, p. 176) gives an analytical proof of the proposition, suggested by an evident theorem in

*line-geometry;*—(using Clifford's expressions): On a quadric 4-spread $Q_{4,5}$ there are two triply infinite systems of planes $R_{2,a}$, $R_{2,b}$; two planes of the same system meet in a point; two of opposite systems in general do not intersect at all, if they do, it is in a line.

There is a similar theorem for quadrics in all flats of odd dimensions.

1. *On a quadric* 2m-*spread* $Q_{2m,2m+1}$ *in* $(2m+1)$*flat* $R_{2m+1}$ *there are two* $\frac{1}{2}m(m+1)$*ply infinite systems of* m-*flats* $R_m$.*

If this holds for $R_{2m-1}$, then it will hold for $R_{2m+1}$. For $R_3$ $(m=1)$ this is the double system of generators $R_1$ on a quadric surface.

Project upon a fixed $r_{2m}$ from a pt. V on the $Q_{2m,2m+1}$. The tangent $R'_{2m}$ at V cuts $Q_{2m,2m+1}$ in a cone-$Q_{2m-1,2m}$, with vertex at V, and it cuts $r_{2m}$ in a fixed $r'_{2m-1}$, which contains the projection through V of the cone-$Q_{2m-1,2m}$ i. e., a fixed $q'_{2(m-1),2m-1}$.

An $R_m$ on the $Q_{2m,2m+1}$ $\left.\begin{matrix}\text{not passing through V}\\ \text{passing through V}\end{matrix}\right\}$ meets the tangent $R'_{2m}$ at V in $\left.\begin{matrix}R_{m-1}\\ R_m\dagger\end{matrix}\right\}$ and is projected into an $\left.\begin{matrix}r_m & \text{having an } r_{m-1}\\ r_{m-1} & \text{lying completely}\end{matrix}\right\}$ on the fixed $q'_{2m-2,2m-1}$ in $r'_{2m-1}$.‡

The converse is also true.§

The $q'_{2(m-1),2m-1}$ is supposed to have on it two $\frac{1}{2}(m-1)m$-ply infinite systems of $(m-1)$flats $r_{m-1}$. In $r_{2m}$ two $r_m$ meet in a point; hence, there are $\infty^m$ $r_m$ through a fixed $r_{m-1}$, i. e., one joining the fixed $r_{m-1}$ to every pt. of the $\infty^m$ pts. of a random fixed $r_m$. These $r_m$ correspond to $R_m$ on the $Q_{2m,2m+1}$.

Therefore, on the $Q_{2m,2m+1}$, there are two $\infty^m \cdot \infty^{\frac{(m-1)m}{2}} = \infty^{\frac{m(m+1)}{2}}$ $=\frac{1}{2}m(m+1)$ply infinite system of $m$-flats $R_m$.

2. *The intersections of the m-flats.*

($k$) $m$ odd.

Two $R_m$ of the same system $(R_{m,a}, R_{m,a})$ in general do not intersect, but in special cases they may intersect in $R_1$, or $R_3$, or . . . or $R_{m-2}$.

Two of opposite systems $(R_{m,a}, R_{m,b})$ in general intersect in a point, but may intersect in $R_2$, or $R_4$, or . . . or $R_{m-1}$.

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* There is on the quadric no $R_s$ if $s > m$; (III, $A_1$).

† If an $R_m$ passes through V, it lies completely in the tangent $R'_{2m}$ at V.

‡ Thus the intersection of an $r_m$ (the projection of an $R_m$) with the fixed $r'_{2m-1}$ lies completely on the fixed $q'$; and likewise two $r_m$ and the fixed $r'_{2m-1}$ intersect on the fixed $q'$.

§ A quadric $m$-spread passing through V would project into an $m$-flat $r_m$ having a quadric $(m-2)$spread on $q'$.

(*l*) $m$ even.

Two $R_m$ of opposite systems in general do not intersect, but may intersect in $R_1$, or $R_3$, or . . . or $R_{m-1}$.

Two $R_m$ of the same system in general intersect in a point, but may intersect in an $R_2$, or $R_4$, or . . . or $R_{m-2}$.

This may be expressed ($R_0 \equiv$ a point, $0 \equiv$ no intersection), beginning with the *most infrequent* cases:

Two $R_m$ of $\left.\begin{matrix}\text{the same}\\ \text{opposite}\end{matrix}\right\}$ system intersect in

$$\left.\begin{array}{lcc} & \text{in general} & \\ & m \text{ even} & m \text{ odd} \\ R_{m-2}, \text{ or } R_{m-4}, \text{ or } \ldots \text{ or} & R_0 \quad , & 0 \\ R_{m-1}, \text{ or } R_{m-3}, \text{ or } \ldots \text{ or} & 0 \quad , & R_0 \end{array}\right\}$$

(1). A few considerations to be used in the proof are given here.

In $r_{2m}$ two $r_m$ meet in a pt. $r_0$; if in another pt., then in a line $r_1$; if two $r_m$ have an $r_s$ common, and also another pt., then they have an $r_{s+1}$ in common.

Two $r_m$ intersecting on $q'$ in $r_s$ may intersect in another point; and thus in $r_{s+1}$; but they do not intersect in *two* other asyzygetic points, for then the intersection with the fixed $r'_{2m-1}$ would not lie entirely on the $q'$. (Cf. foot-note ‡, §1.)

If two $r_m$ intersect only in an $r_s$ lying on the $q'$, the two corresponding $R_m$ meet the $R_{s+1}$ joining this common $r_s$ to the fixed point V in two $R_s$ (which were both projected into the common $r_s$) which in the $R_{s+1}$ intersect in an $R_{s-1}$. As a special case, if two $r_m$ intersect in a *point* $r_0$ on $q'$, the two corresponding $R_m$ [intersect the line $R_1$, joining $r_0$ to V, in two points $R_0$ and] do not intersect.

(2) $m$ even. The $r_{m-1}$ on $q'$ intersect according to ($k$), $m-1$ being odd.

On $q'$ $r_{m-1,a}$ and $r_{m-1,a}$ in general do not intersect, but may intersect in $r_1, r_3$ . . . . or $r_{m-3}$. Two $r_m$ through them must intersect in a point, at least. Hence, in general, $m$ being even, two $R_m$ of the same system intersect in a point. In the particular cases, if the two $r_m$ intersect entirely on $q'$, the two $R_m$ intersect in $R_0, R_2$ . . . or $R_{m-4}$; but if the two $r_m$ intersect also in a point not on $q'$, the two $R_m$ intersect in $R_2, R_4$ . . . or $R_{m-3}$.

On $q'$ $r_{m-1,a}$ and $r_{m-1,b}$ intersect in a point $r_0$; but may intersect in $r_2, r_4$ . . . or $r_{m-2}$. Two $r_m$ through them would in general intersect in no external point; hence, in general, $m$ being even, two $R_m$ of opposite systems do not intersect. In the particular cases, if the two $r_m$ intersect entirely on $q'$, the two $R_m$ intersect in $R_1, R_3$ . . . or $R_{m-3}$; but if the $r_m$ intersect also in a point not on $q'$, the two $R_m$ intersect in $R_1, R_3$ . . . or $R_{m-1}$.

Thus, the $(k)$ holding for $m-1$ odd, the $(l)$ hold for $m$ even.

(3) $m$ odd. By similar considerations it is shown that, the $(l)$ holding for $m$ even, the $(k)$ hold for $m+1$ odd. But the $(k)$ hold for $m=1$ on the quadric $Q_{2,3}$ in $R_3$ (as might be shown immediately by the projection from V on a plane); and, therefore, $(k)$ and $(l)$ are true in general.

3. One $m$-flat of each system passes through every $R_{m-1}$ of the $Q_{2m}$. The $R_{m-1}$ projects into an $r^{m-1}$ having an $r_{m-2}$ on $q'$, through which (suppose) there pass an $r_{m-1,a}$ and an $r_{m-1,b}$; the original $r_{m-1}$ and the $r_{m-1,a}$, intersecting in $r_{m-2}$, determine an $r_m$ the projection of an $R_{m,a}$ through the $R_{m-1}$. This is true on the $Q_{2,3}$; therefore, in general.

Every pt. in $R_{2m+1}$ has a polar $R_{2m}$ with reference to the $Q_{2m}$; the polar $R_{2m}$ of every pt. in an $R_s$ passes through a certain $R_{2m-s}$ which is called the polar of the $R_s$. (Clifford.)

If the $s$-flat $R_s$ lie on the $Q_{2m}$, the polar $R_{2m}$ of every point in it, i. e., the tangent $R_{2m}$ at that point, passes through the $s$-flat itself; hence the polar $P_{2m-s}$ must include the original $s$-flat $R_s$.

The $m$-flats $R_m$ are *self-polar.*

The $R_{m,a}$ and $R_{m,b}$ through an $R_{m-1}$ taken together lie in and determine the polar $R_{m+1}$ of the $R_{m-1}$.

More generally, two $R_m$ intersecting in an $R_s$ lie in and determine the polar $R_{2m-s}$ of the $R_s$.

## III.—On Knots, with a Census for Order Ten. By C. N. Little, Lincoln, Neb.

1. Gauss in 1833* called attention to the importance of the study of the ways in which cords might be linked. Nothing, however, appears to have been written upon the subject until in 1847 Listing published his *Vorstudien zur Topologie.*† In this he briefly but in a masterly way touched upon the subject of knots, established some of the fundamental propositions, and proposed a notation which, as slightly modified by Prof. Tait, furnishes the point of view for the present paper. In a communication‡ to Prof. Tait in 1877, Listing points out the fragmentary character of his own contribution to the subject, and says that the type-symbol used by him is "nichts weiter als ein derartiger Fingerzeig."

It is to Professor Tait, however, that the greater part of our present knowledge of the subject is due. He, independently of Listing, obtained the fundamental propositions and found the knots and their forms for orders from three to seven inclusive.§

In 1884 Kirkman‖ published the forms of knots of orders eight and nine, and immediately Tait, making use of Kirkman's work, extended his census of knots to these orders.¶

2. Professor Tait has shown that any closed plane curve of $n$ crossings divides its plane into $n+2$ compartments; that these compartments are in two groups; that, at the crossings, like compartments are vertically opposite. We shall call these compartments of the plane *parts.* A part is represented by the number equal to the number of double points on its perimeter. The sum of the numbers representing the parts of either group is $2n$, that is, these numbers together constitute a partition of $2n$. The partitions for the two groups together make up Listing's *type-symbol.* As it can lead to

* "Eine Hauptaufgabe aus dem Grenzgebiet der *Geometria Situs* und der *Geometria Magnitudinis* wird die sein, die Umschlingungen zweier geschlossener oder unendlicher Linien zu zählen."—Werke. Göttingen. 1867, vol. v, p. 605.

† Göttingen Studien, 1847. I have been able to see only Tait's apparently full abstract in Proc. Roy. Soc. Edin., vol. ix, pp. 306–309.

‡ Proc. Roy. Soc. Edin., vol. ix, p. 316.

§ On Knots, Trans. Roy. Soc. Edin., xxviii, 145–191, 1876–77

‖ Trans. Roy. Soc. Edin., xxxii, 281–309.

¶ Trans. Roy. Soc. Edin., xxxii, 327–342

no ambiguity we shall also call the number representing a compartment a *part*, and either group of compartments a *partition*.

Since every closed plane curve of $n$ crossings, having double points only, may be read alternately over and under at the crossings, every such curve which gives parts, none greater than $n$ or less than 2, may be taken as a projection of a reduced knot of $n$ crossings. We call such curves *knot-forms* or briefly, *forms*, and regard two forms as distinct if they do not have the same parts similarly arranged.

The first part of the problem is to find all the different knot-forms of any order.

Since the same knot may be transformed so as to be projected into more than one knot-form, the second part of the problem is, from the complete series of knot-forms of $n$ crossings to find all the different $n$-fold knots. Knots exist for which the law of over and under does not hold; these are not considered in the present paper.

3. It is unnecessary to do more than allude to two very distinct and very ingenious methods devised and used, the one by Tait and the other by Kirkman, for the solution of the first part of this problem. We may perhaps infer from Professor Tait's opinion* that "a full study of 10-fold and 11-fold knottiness seems to be relegated to the somewhat distant future," that they were more laborious than proves to be necessary.

4. A third method, based on Listing's type-symbol, is thus described by Professor Tait at page 168 of his first memoir.

"Write all the partitions of $2n$, in which no one shall be greater than $n$ and no one less than 2. Join each of these sets of numbers into a group, so that each number has as many lines terminating in it as it contains units. Then join the middle points of these lines (which must not intersect one another), by a continuous line which *intersects* itself at these middle points and there only. When this can be done we have the projection of a *knot*. When more continuous lines than one are required we have the projection of a linkage."

On page 160 of the same memoir, he says, speaking of this method: "But we can never be quite sure that we get *all* possible results by a semi-tentative process of this kind. And we have to try an immensely greater number of partitions than there are knots, as the great majority give links of greater or less complexity."

It seems possible however, with the help of some simple theorems to make the "Partition Method" exhaustive, and wholly to do away with the drawing of links.

* In 1884. Trans. R. S. E., xxxii, 328

5. An inspection of form A*a* of Plate I will make clear some terms already introduced and others that we shall now require. Regarding the curve A*a* as alone in a plane, it divides it into twelve *parts*, two 9-gons, two 3-gons and eight 2-gons. The external 3-gon or *amplexum* differs in no way from the other parts. Of these twelve parts, two 9-gons and one 2-gon form one group—the *leading* partition; the two 3-gons and seven 2-gons form the other group—the *subordinate* partition. The terms leading and subordinate are relative merely, but that partition will be taken as leading which has the smaller number of parts. The *type-symbol* for A*a* is $\left\{\begin{matrix} 9^2 2 \\ 3^2 2^7 \end{matrix}\right.$.

6. The double points common to the perimeters of two parts of the same partition will be called *bonds* of those parts, and the parts are said to be bound by these bonds. It is well known that a type-symbol does not determine a form. For this, it is necessary to know the numbers of bonds between the several parts of either partition, together with the arrangement of these parts.

In general the parts of a given partition may be bound in more than one way giving forms that may be projections of either links or knots. Each set of numbers of bonds of the several parts of the given partition is a *clutch* of that partition.

The *class* $p$ of a partition is the number of parts in it. The class of a form is the class of its leading partition. The *order* of any partition is equal to $n$ and is the same as the order of all knot-forms derivable from it. The *deficiency* $\kappa$ of a partition is its order minus its greatest part.

7. Let the parts of $2n$ be A, B, C, . . . P arranged in order of magnitude, and the numbers of bonds of each part be respectively $\alpha, \beta, \gamma, \ldots \pi$. Let the number of bonds common to any two parts as A and B be (AB). Then

$$\left.\begin{array}{l} (AB)+(AC)+\ldots\ldots\ldots(AP)=\alpha \\ (AB)+(BC)+\ldots\ldots\ldots(BP)=\beta \\ (AC)+(BC)+\ldots\ldots\ldots(CP)=\gamma \\ \quad\cdot\quad\cdot\quad\cdot\quad\cdot\quad\cdot\quad\cdot \\ (AP)+(BP)+\ldots\ldots\ldots(OP)=\pi \end{array}\right\}(a)$$

or $n$ equations with $\frac{1}{2}n(n-1)$ unknown quantities which can have only positive integral values. The possible solutions of $(a)$ will evidently give all the clutches for this partition.

8. I. THEOREM.—If a part be solely bound to a second part, or if any $q$ parts ($q \not> p-2$) be bound mutually in any way and all free bonds of these parts go to a single part, then this portion of the form constitutes a separate knot (unless there be linkage) and the string concerned in it may be drawn tight without affecting the remainder of the knot-form. Such knots are not considered as belonging to order $n$.

In particular a 2-gon so bound throws out from consideration a clutch.

9. II. THEOREM.—No knot-form of the $n$th order has as leading partition one whose class exceeds $\varkappa+2$.

Adding equations ($a$) above, dividing by two, and subtracting the first and any other, say the second, we find

$$-(\mathrm{AB})+(\mathrm{CD})+(\mathrm{CE})+\ \ldots\ (\mathrm{CP})+\ \ldots\ (\mathrm{OP})=n-\alpha-\beta,$$
$$=\varkappa-\beta.$$

Therefore, $(\mathrm{AB}) \not< \beta-\varkappa$.

In a similar way

$$(\mathrm{AC}) \not< \gamma-\varkappa$$
$$(\mathrm{AD}) \not< \delta-\varkappa$$
$$\cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot$$
$$(\mathrm{AP}) \not< \pi-\varkappa$$

Adding $(\mathrm{AB})+(\mathrm{AC})+\ \ldots\ (\mathrm{AP}) \not< \beta+\gamma+\ \ldots\ \pi-(p-1)\varkappa$

$$\not< n+\varkappa-(p-1)\varkappa$$
$$\not< n-(p-2)\varkappa,$$

we have then the two conditions

$$\left.\begin{aligned}(\mathrm{AB})+(\mathrm{AC})+\ \ldots\ (\mathrm{AP}) &\not< n-(p-2)\varkappa\\ &=n-\varkappa.\end{aligned}\right\}(b)$$

Now suppose, if possible, $p=\varkappa+3$

$$(\mathrm{AB})+(\mathrm{AC})+\ \ldots\ (\mathrm{AP})=n-\varkappa \qquad =n-\varkappa$$
$$\not< n-(\varkappa+1)\varkappa \quad \not< n-\varkappa-\varkappa^2.$$

To the minimum values of (AB), (AC), etc., (that is, to $\beta-\varkappa$, $\gamma-\varkappa$, . . . ) must be added $\varkappa^2$ in all, and to no one more than $\varkappa-1$, by I. The $\varkappa+2$ smallest parts of $\varkappa$ square are evidently $(\varkappa-1)^4$ $(\varkappa-2)^{\varkappa-2}$. By adding these $\varkappa+2$ parts in any way to the minimum values, a clutch will be given in which each of four parts will have a single bond not going to A, and each of $\varkappa-2$ parts will have two. A part of the latter kind cannot have its two free bonds carried to a second part of the same kind, by I. If two parts be joined by a single bond there will be left two free bonds. Ultimately it will be necessary to join two parts of the first kind to a combination of parts having but two free bonds, and I will apply. If any of the $\varkappa+2$ parts of $\varkappa^2$ be diminished by $s$ then will $s$ parts of $2n$ be

added to those of the first kind, and however the free bonds may be arranged, ultimately the same result as before will be reached. Therefore $p$ cannot equal $\varkappa+3$ and still give knot-forms.

Much less can $p$ be greater than $\varkappa+3$.

10. A given clutch of a leading partition does not uniquely determine a form. The following proposition however holds.

III. THEOREM.—All or none of the forms determined by any given clutch of a partition are knot-forms of the order considered.

For, all forms to be had from any clutch of a given partition may be obtained by taking all the possible different changes (consistent with the given clutch) of relative position of the various parts. But these can all be effected by successive interchanges of the connections of two parts, whether such connections are direct (by a single bond), by a 2-gon, by a 3-gon, or are more complicated. We may therefore confine the attention to a definite portion of the knot and keep the remainder fixed. Let A and B, (Fig. 1, Plate I), be two parts connected as shown. Two strings, or two parts of a single string, are involved. If there were more all but two would be closed. Let the ends of these strings, or parts of a single string, leave the portion of the form under consideration at $a$ and $c$ on the perimeter of A, and $b$ and $d$ on that of B. Cut at these points and call the ends $a$ and $a'$, $b$ and $b'$, $c$ and $c'$, $d$ and $d'$; $a$, $b$, $c$ and $d$ remain fixed. Now revolve through 180° about the axis AB, and join the free ends.

Before the change there may be three cases. The strings may be $\begin{cases} c\,d \\ a\,b \end{cases}$ $\begin{cases} b\,d \\ a\,c \end{cases}$ or $\begin{cases} b\,c \\ a\,d \end{cases}$ After the change $a'$ is joined to $c$, and $c'$ to $a$; $b'$ to $d$, and $d'$ to $b$.

$$\begin{cases} c\,d \\ a\,b \end{cases} \text{ become } \begin{cases} c\,a'\,b'\,d \\ a\,c'\,d'\,b \end{cases}$$
$$\begin{cases} b\,d \\ a\,c \end{cases} \text{ " } \begin{cases} b\,d'\,b'\,d \\ a\,c'\,a'\,c \end{cases}$$
$$\begin{cases} b\,c \\ a\,d \end{cases} \text{ " } \begin{cases} b\,d'\,a'\,c \\ a\,c'\,b'\,d \end{cases}$$

Therefore coming up to this part of the knot on any string, we must leave on the same string before and after the change. If then the form was a knot before the change it will be one after, and if a link before it will be a link after.

11. *Coils.*—A succession of $n$ 2-gons constitutes an *n-coil*, which may be open or closed. Since at the 3rd or $2n+1$st crossing of a coil the strings have the relative position of the first crossing, if the coil be closed by carrying around the ends to the beginning and

joining them so as to preserve the law of over and under one string will be formed. While if from the $2n$th crossing the strings are carried around, that string over at the 1st is under at the $2n$th and, on joining, there will be two strings.

Hence, as is well known, $\begin{matrix}(2n+1)^2\\(2)^{2n+1}\end{matrix}$ is always a knot, while $\begin{matrix}(2n)^2\\(2)^{2n}\end{matrix}$ is always a link.

12. For the purpose of distinguishing between clutches giving knots and those giving links, it follows from Theorem III that we may take the direct bonds between any two parts together, and these form open coils of the subordinate partition; and further it is evident from § 11 that any odd open coil (even number of bonds), may be dropped, and any even coil (odd number of bonds) may be replaced by a single bond. If the resulting clutch gives link, so would the original. If the resulting clutch be still too complex for easy recognition of its character, the clutch of the subordinate partition of the resulting form may perhaps be still farther reduced in the same way. If the clutches of lower orders were at hand they also could be used for settling the question.

13. We have the following theorems for throwing out clutches unproductive of knot forms.

IV. Theorem.—If a part be joined to other parts in every case by an even number of bonds, there is linkage. For, the string about this part is closed by Section 12.

V. Theorem.—If two parts are connected by two 2-gons (of the same partition with the parts) there is linking. For they may be put in succession by Theorem III. When this is done there is a 4-gon of the negative partition bound to two other parts in each case by two bonds, and IV applies.

VI. Theorem.—An odd part joined to one part by an odd number of bonds and to other parts in every case by an even number of bonds may be dropped; for, by Theorem III it becomes a loop with a single crossing, and this can have no effect on the question of linking.

In particular a 3-gon joined by one bond to one part and by two bonds to a second part may be dropped.

If two odd parts are joined by an odd number of bonds, and are joined to other parts in every case by an even number of bonds there is linkage.

In particular two 3-gons so joined throw out the clutch.

VII. Theorem.—If two 3-gons, C and D, are themselves joined directly and are joined to A and B in each case by a single bond there is linkage.

For, HCM (fig. 2, Plate I) under we will say at C is over at H and M. LCN is then over at C and under at L and N. LHN is over at L, under at H, and over at N. Its continuation is therefore NML which is over at N, under at M, and over at L.

14. Class III, Order $n$.—In this class we have

$$(AB)+(AC)+(BC)=n$$

and an unique solution of equations ($a$), § 9. Therefore $(BC)=\varkappa$, $(AB)=\beta-\varkappa$ and $(AC)=\gamma-\varkappa$. If two or three of these quantities be even, the clutch to which they belong will give a linkage, by Theorem IV. In other cases by § 12 there is a knot.

Suppose $n$ to be odd and $\alpha$ even; then $\varkappa$ is odd, and $\beta$ and $\gamma$ are both odd, or both even. In the first case the clutch gives a link, in the second a knot.

Suppose $n$ to be odd, and $\alpha$ odd; then $\varkappa$ is even, and of $\beta$ and $\gamma$ one must be odd and the other even. The clutch gives a link.

Suppose $n$ to be even and $\alpha$ even; then $\varkappa$ is even and $\beta$, $\gamma$ are both odd or both even. In the first case a knot, in the second a link.

Suppose $n$ to be even and $\alpha$ odd; then $\varkappa$ is odd, and of $\beta$ and $\gamma$ one must be odd and one even, and there is a knot. This proves the following:

VIII. Theorem.—In odd orders only partitions of $2n$ into three even parts, give knots, while in even orders, only these partitions give links.

15. Class IV, Order $n$. Here

$$\begin{aligned} -(AB)+(CD)&=\varkappa-\beta \\ -(AC)+(BD)&=\varkappa-\gamma \\ -(AD)+(BC)&=\varkappa-\delta \\ (AB)+(AC)+(AD)&\begin{matrix}=n-\varkappa \\ \not< n-2\varkappa\end{matrix} \end{aligned}$$

The minimum values of (AB), (AC), (AD), (BC), (BD), (CD), are respectively $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, 0, 0, 0. To get every clutch we must add in every possible way to the minimum values of (AB), (AC), (AD), all the partitions of $\varkappa$ into not more than three parts, none greater than $\varkappa-1$. But evidently we must increase the minimum values of any quantity of the second set (BC), (BD), (CD), by the same number that we increase the corresponding quantity of the first set. The following scheme which considers in detail every possible case, expresses clearly the propositions for determining whether clutches of partitions of this class furnish knots or links. Let e or o indicate whether a number be even or odd.

| $n$, | $\kappa$ | Partition. | Add $\kappa$= | $\beta-\kappa$, $\gamma-\kappa$, $\delta-\kappa$. | AB. AC. AD. | BC. BD. CD. | Form. | Props. or Sect. used. |
|---|---|---|---|---|---|---|---|---|
| e | e | e e e e | e e e | e e e | e e e | e e e | Link. | IV. |
| | | | e o o | | e o o | o o e | " | §12, V. |
| | | e e o o | e e e | e o o | e o o | e e e | " | IV. |
| | | | e o o | | e e e | o o e | " | IV. |
| | | | o e o | | o o e | o e o | Knot. | §12, VI §11. |
| | o | o o o o | o o o | e e e | o o o | o o o | Link. | §12, VII. |
| | | | o e e | | o e e | e e o | " | §12, VI. |
| | | o o e e | o o o | e o o | o e e | o o o | Knot. | §12, VI, 11. |
| | | | o e e | | o o o | e e o | " | §12, VI, 11. |
| | | | e o e | | e e o | e o e | Link. | IV. |
| o | e | o o e e | e e e | o e e | o e e | e e e | " | IV. |
| | | | e o o | | o o o | o o e | " | §12, V. |
| | | | o e o | | e e o | o e o | Knot. | §12. |
| | e | o o o o | e e e | o o o | o o o | e e e | " | §12, |
| | | | e o o | | o e e | o o e | " | §12. |
| | o | e e e e | o o o | o o o | e e e | o o o | Link. | IV. |
| | | | o e e | | e o o | e e o | " | IV. |
| | | e e o o | o o o | o e e | e o o | o o o | " | §12, V. |
| | | | o e e | | e e e | e e o | " | IV. |
| | | | e o e | | o o e | e o e | Knot. | §12. |

The first line of this scheme says that when $n$ is even and $\kappa$ even, and $2n$ divided into four even parts, the minimum values of (AB), (AC), (AD), will be even, and that if a partition of $\kappa$ into three even parts be added to $\beta-\kappa$, $\gamma-\kappa$, $\delta-\kappa$ the numbers constituting the clutch will be even, and the form a link by Prop. IV.

One of the propositions proved in this scheme is worthy of separate enunciation.

IX. THEOREM.—In even orders partitions of $2n$ into four even or four odd parts give link-forms only. In odd orders partitions into four even parts give link-forms only.

16. X. THEOREM.—In all orders partitions of six even parts give link-forms only.

For, an even number may be divided into five parts, of which four, two or none shall be odd. After the application of §12 the only parts to be found in the leading partition will be 4-gons and 2-gons. Every form under consideration will be reduced so as to have as leading partition one of the following, with a clutch of which every number is 1.

$$4^6,\quad 4^5 2,\quad 4^4 2^2,\quad 4^3 2^3,\quad 4^2 2^4,\quad 4 2^5,\quad 2^6.$$

In the lower orders $4^3 2^3$, $4^2 2^4$, $4 2^5$, and $2^6$ have been found to give no knots.

If the form given by $4^6$ be drawn it is found in any particular case to consist of four closed curves, and therefore by Theorem III is always a link-form. On drawing $4^4 2^2$ it also proves to be a linkage.

The partition $4^{\bullet}2$ with the given clutch can not exist in a plane. This happens in two cases in order 10. On drawing the forms in such cases additional crossings will be found to be necessary. It is therefore true that in all orders, partitions of six even parts give link-forms only.

A synthetic proof of this theorem is possible.

17. Instead of continuing the consideration of the general subject we shall now illustrate the method of determining the knot-forms of any order by a particular consideration of order 10.

The number of partitions of 20 into parts none greater than 10 or less than 2 is 107.* These, arranged in dictionary order, are given in full in Table I.

Of these the single partition of Class II gives a link, § 11; the thirty-seven marked II are cut out by Theorem II. In Class III, Theorem VIII throws out $8^2 4$ and $86^2$; in Class IV, Theorem IX the eight partitions marked IX; and in Class X, Theorem X the three marked X. The partitions remaining in Classes III to VI inclusive are alone taken as leading partitions; for, all remaining partitions appear in every possible way as subordinate partitions (§ 2), and can, therefore, furnish no additional knot-forms.

We have then to tabulate the clutches of those partitions still remaining in Classes III to VI. We at once write down Tables II and III in which are omitted all clutches that are thrown out by Sections 14 and 15. In Class V, a table with headings as shown in Table IV is used. We take for illustration the first partition, $7^2 2^2$. Here $\varkappa=3$, and $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, $\varepsilon-\varkappa$ are 4, $-1$, $-1$, $-1$. In this class we must add in every possible way to the minimum values of (AB), (AC), (AD), (AE) the partitions of $2\varkappa$ into four parts, or fewer, none greater than $\varkappa-1$. The only partitions of 6 meeting these conditions are $2^3$ and $2^2 1^2$, which may be added in seven different ways to 4, $-1$, $-1$, $-1$, giving the different clutches of the table. Thus adding 1, 2, 2, 1, to $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, $\varepsilon-\varkappa$ we have (AB), (AC), (AD), (AE), equal to 5, 1, 1, 0, respectively. Subtracting (AB) from $\beta$, etc.

$$\Sigma B = (BC) + (BD) + (BE) = 2$$
$$\Sigma C = (BC) + (CD) + (CE) = 1$$
$$\Sigma D = (BD) + (CD) + (DE) = 1$$
$$\Sigma E = (BE) + (CE) + (DE) = 2.$$

These quantities are put in the columns headed $\Sigma B$, $\Sigma C$, $\Sigma D$, $\Sigma E$,

* See Tait, Trans. Roy. Soc. Edin., vol. xxxii, p. 342.

and all the possible solutions of this set of equations here as throughout the work are written down by inspection. The rest of this table is made out in the same way. In Table V we must, in every possible way, add the partitions of $3\varkappa$ into four parts or fewer, none greater than $\varkappa-1$. In the general case we add the partitions of $(p-3)\varkappa$ into $p-1$ parts none greater than $\varkappa-1$. In the complete Table IV there are 400 clutches, and in Table V 1000. It has not been thought necessary to publish more than a sample of either table.

18. Having completed the tables of clutches we next cast out in Tables IV and V all clutches unproductive of knot-forms. The theorems already established are sufficient for this purpose. The routine followed will be readily understood from considering its use in the samples given of Tables IV and V.

Partition $7^2 2^3$: in clutch (1) A and B are connected by two 2-gons, and V applies; (4) becomes (3) by interchanging D and E; (7) becomes (6) by interchanging C and D; and in (2) and (5) we have a 2-gon solely bound to a single part, and I applies.

Partition $6^2 42^2$: clutch (1) is thrown out by IV since a 6-gon B is joined to A by four bonds and to C by two; Theorem IV casts out (4) because of C, (14) because of C, (17) because of B, and (20) because of A; in (2), (3), (8), (9), (11), (12), (15) and (18) a 2-gon is solely joined to a single part, and I applies; in (5) B and C form a combination solely bound to A, and I applies; I throws out also (10) where D and E are bound together and to C, and (13) where the same parts are bound to B; interchanging D and E (7) becomes (6); interchanging A and B (16) becomes (7).

Partition $5^3 32$: I throws out (2), (3), (6), (8), (14), (16), (19), (20), (26), (27), (30), (31), (33), (37) and (38); by interchanges of parts

| | | | |
|---|---|---|---|
| (5)≡(4), | (17)≡(5), | (24)≡(9), | (32)≡(11), |
| (7)≡(1), | (18)≡(3), | (25)≡(4), | (34)≡(12), |
| (13)≡(10), | (21)≡(10), | (28)≡(23), | (35)≡(1), |
| (15)≡(12), | (22)≡(21), | (29)≡(23), | and (36)≡(9). |

In (4) D is dropped by VI, and B thus is changed to a 4-gon; then the application of § 12 leaves the two 3-gons A and C joined by two 2-gons, E and the still farther reduced B; V, therefore, shows that the clutch gives links only. In (9), drop C by VI and B becomes a 2-gon and A a 3-gon; the two 3-gons A and D are connected by the two 2-gons E and the reduced B; therefore the clutch gives links, by V. In (11) drop C by VI and the two 3-gons A and B are connected by the two 2-gons E and the reduced D; V applies.

In Table V, clutch No. (1) of $53^5$ is thrown out by I. In (2) the two 3-gons E and F are joined together by a single bond and to A in each case by two bonds, and VI applies; or we might use I. Theorem VI throws out (4), since the two 3-gons B and F are joined to each other by a single bond and to other parts in each case by two bonds. In (5), drop F, by VI; B becomes a 2-gon joining D and E, and they are 3-gons connecting A and C. An obvious extension of Theorem VII throws out the clutch. Since in (7) B and C are joined together and to A, I applies.

This process is continued until all clutches which do not give knot-forms have been thrown out, as well as those clutches which are repetitions of clutches previously given. The samples given of Tables IV and V constitute about one-twentieth of the complete tables.

19. We are now ready to draw the forms which the remaining clutches furnish.

Definition.—The number of *Circular arrangements* of $n$ things is the number of distinct ways in which $n$ things can be arranged in a circle, the order whether direct or retrograde being of no consequence.

For illustration of the general method we consider in detail the productive clutches of partition $6^2 42^2$. In clutch (6) the two 6-gons are connected, by three bonds, by a 2 gon, and by a 4-gon which is connected with one 6-gon by two bonds and with the other by a 2-gon and one bond. There will be as many forms from this clutch as there are circular arrangements of a a a b (cd), where c and d may not be separated. These are

a a a b (cd)
a a a b (dc)
a a b a (cd)
a a b a (dc).

The four forms $C^2c_1$, $C^2c_2$, $C^2c_3$, $C^2c_4$, can at once be drawn. See Plate V, Knot LXIV.

In (19), the only other clutch of this partition that affords forms, a 6-gon is connected with a 4-gon directly, by a 2-gon, and by a 6-gon which is connected with the 4-gon by a single bond and by a 2-gon. There are two circular arrangements of a b (cd), namely a b (cd) and b a (cd); these furnish the two forms $C^2d_1$, $C^2d_2$. Knot XXX, Plate III. In practice the operations described in this section and in the preceding are performed simultaneously.

20. In Class VI every productive partition is used as a leading

partition. But since the subordinate partitions here belong to the same class, every form, with certain exceptions, will be found twice; this affords a check on the completeness of this part of the work. The exceptions are the *amphicheiral* knot-forms. These have the same partitions similarly connected both as leading and as subordinate partitions. These therefore appear but once.

In the partition $53^5$, which was given as the shortest possible illustration of Table V, clutch (3) furnishes a single form $D^2s_2$, which already had been obtained under $54^2 32^2$; clutch (6) gives two which had been obtained, $Du_1$ under $643^2 2^2$ and $D^2x_4$ under $54^2 32^2$. Clutch (8) gives the only new knot-form from this partition, viz: the amphicheiral $D^2o$.

From the clutches of Classes III, IV, V and VI 364 ten-fold knot forms are obtained.

21. *The Derivation of Knots from Knot-Forms.*—Prof. Tait has not described the methods which he used in his derivation of the knots of lower orders from the knot-forms. In 1884 he says:* "the treatment to which I have subjected Kirkman's collection of forms, in order to group together mere varieties or transformations of one special form, is undoubtedly still more tentative in its nature; and thus, though I have grouped together many widely different forms, I cannot be *absolutely* certain that all those groups are essentially different from one another."

If a ten-fold knot be placed upon a plane in such a way as to have but ten crossings the eye will project it upon the plane in a form which will be found among the 364 above obtained. If the knot gives more than one form it will be possible to obtain any other of its forms by one or more turnings over of restricted portions of the knot while the remainder is held fixed. Now the string cannot issue from the portion of the knot that is turned at more than four points, for in that case the turning would introduce consecutive overs, and one or more additional crossings; the portion of the knot that is turned must therefore be wholly between two parts of the given knot form and in turning it we untwist two of the strings at one point and twist two at another, the result being simply to change the position of a single bond from one end of the connection to the other. The class of the form is therefore not changed, and all the forms of any knot belong to the same class.

22. Moreover in order 10 and lower orders all the forms coming from any clutch are obtained by changing the position of single

* Trans. Roy. Soc. Edin., vol. xxxii, p. 327.

bonds in the connections of pairs of parts. Therefore in these orders all forms from any clutch are forms of the same knot. The subordinate partitions of these forms are then to be examined and all forms added which are obtained from them by changing the positions of connections of their parts, retaining the given clutch of the subordinate partition. These forms in turn are treated in the same way, but it will usually happen that no new form of the knot is obtained and the complete determination of the knot in all of its forms is finished.

23. We take for illustration Knot I of Class IV, shewn on Plate I. $Ba_1$ becomes $Ba_2$ by twisting about a vertical axis the 2-gon connecting the 8-gon and 7-gon. The first crossing below is opened and the strings above are crossed, the rest of the knot remaining fixed. Twisting the 2-gon again $Ba_3$ is obtained and nothing new is gotten by further changes of the forms. Since the negative partition in every case consists of two parts joined by three symmetrical connections, which have only one circular arrangement, there are no other forms of Knot I.

24. The knots of Class III, order $n$, are unique; since three things have but one circular arrangement.

25. The knots of Classes III, IV, V will be found with their forms grouped together in Plates I–V. On Plates V–VII are figured the forms of Class VI grouped as they come from the clutches, except that no form is repeated. The knots of this class will be found in Table VI. Every knot-form is the projection of two knots, one of which is the perverted image of the other, and consequently each group of knot-forms belongs to two knots which are in general different. If a series of knot-forms contains any amphicheiral form then it will also contain the perversion of every form of the series not amphicheiral. The series consists of the forms of one knot and not of two.

26. In order 10 I find, counting a knot and its irreconcilable perversion as two:

| Class. | Forms. | Knots. | Knots. |
|---|---|---|---|
| III, | 6 | 12 | 6 |
| IV, | 25 | 30 | 15 |
| V, | 200 | 128 | 64 |
| VI, | 133 | 64 | 39 |
| Totals, | 364 | 234 | 124 |

In lower orders Professor Tait has found:

| Orders. | Forms. | Knots. | Knots. |
|---|---|---|---|
| 3 | 1 | 2 | 1 |
| 4 | 1 | 1 | 1 |
| 5 | 2 | 4 | 2 |
| 6 | 3 | 5 | 3 |
| 7 | 10 | 14 | 7 |
| 8 | 27 | 31 | 18 |
| 9 | 100 | 82 | 41 |

The fourth column contains the numbers as they are given by Tait, the perversions of knots not being counted.

In so long a labor as is involved in making such a census the opportunities for error are many. Any errors or omissions that may be found in the census are to be attributed to the writer rather than to the method, which is simple and direct.

TABLE I.—*Partitions of* 20.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CLASS II. | | CL. IV.—Contin'd | | CL. V.—Contin'd. | | CLASS VI. | CL. VII.—Conti'd |
| | $10^2$ | IX. | $8^2 2^2$ | II. | $86^2 3^3$ | II. | $102^5$ | II. | $642^5$ |
| CLASS III. | | | 8732 | | $8532^2$ | II. | $932^4$ | | $63^2 2^4$ |
| II. | 1082 | IX. | 8642 | | $84^2 2^2$ | II. | $842^4$ | | $5^2 2^5$ |
| | 1073 | | $863^2$ | | $843^2 2$ | | $83^2 2^3$ | | $5432^4$ |
| | 1064 | | $85^2 2$ | | $83^4$ | | $752^4$ | | $53^3 2^3$ |
| | $105^2$ | | 8543 | | $7^2 2^3$ | II. | $7432^3$ | | $4^3 2^4$ |
| | $9^2 2$ | IX. | $84^3$ | | $7632^2$ | | $73^2 2^2$ | | $4^2 3^2 2^3$ |
| | 983 | | $7^2 42$ | | $7542^2$ | X. | $6^2 2^4$ | | $43^4 2^2$ |
| | 974 | IX. | $7^2 3^2$ | | $753^2 2$ | | $6532^3$ | | $3^6 2$ |
| | 965 | | 7652 | | $74^2 32$ | X. | $64^2 2^3$ | | |
| VIII. | $8^2 4$ | | 7643 | | $743^3$ | | $643^2 2^2$ | CLASS VIII. | |
| | 875 | IX. | $75^2 3$ | | $6^2 42^2$ | | $63^4 2$ | II. | $62^7$ |
| VIII. | $86^2$ | | $754^2$ | | $6^2 3^2 2$ | | $5^2 42^3$ | II. | $532^6$ |
| | $7^2 6$ | IX. | $6^3 2$ | | $65^2 2^2$ | | $5^2 3^2 2^2$ | | $4^2 2^6$ |
| CLASS IV. | | | $6^2 53$ | | 65432 | | $54^2 32^2$ | | $43^2 2^5$ |
| II. | $1062^2$ | IX. | $6^2 4^2$ | | $653^2$ | | $543^3 2$ | | $3^4 2^4$ |
| | 10532 | | $65^2 4$ | | $64^3 2$ | | $53^5$ | CLASS XI. | |
| | $104^2 2$ | IX. | $5^4$ | | $64^2 3^2$ | X. | $4^4 2^2$ | II. | $42^8$ |
| | $1043^2$ | CLASS V. | | | $5^3 32$ | | $4^3 3^2 2$ | | $3^2 2^7$ |
| II. | $972^2$ | II. | $1042^3$ | | $5^2 4^2 2$ | | $4^2 3^4$ | CLASS X. | |
| | 9632 | | $103^2 2^2$ | | $5^2 43^2$ | CLASS VII. | | | $2^{10}$ |
| | 9542 | II. | $952^3$ | | $54^3 3$ | II. | $82^6$ | | |
| | $953^2$ | | $9432^2$ | | $4^5$ | II. | $732^5$ | | |
| | $94^2 3$ | | $93^3 2$ | | | | | | |

TABLE II.—*Clutches for* $p=3$.

| Partition. | (AB) (AC) (BC) | Partition. | (AB) (AC) (BC) | Partition. | (AB) (AC) (BC) |
|---|---|---|---|---|---|
| $9^2 2$ | 8 1 1 Aa | 974 | 6 3 1 Ac | 875 | 5 3 2 Ad |
| 983 | 7 2 1 Ab | 965 | 5 4 1 Ae | $7^2 6$ | 4 3 3 Af |

TABLE III.—*Clutches for $p=4$.*

| Partition. | $\beta-\kappa$ $\gamma-\kappa$ $\delta-\kappa$ | (AB) (AC) (AD) | (BC) (BD) (CD) | (ΣB) (ΣC) (ΣD)* | |
|---|---|---|---|---|---|
| 8732 | 5 1 0 | 5 2 1 | 1 1 0 | 2 1 1 | $Ba_1$, $Ba_2$, $Ba_3$. |
| $\kappa=2$ add 1, 1 | | 6 1 1 | 1 0 1 | 1 2 1 | Bb. |
| 8633 | 4 1 1 | 5 1 2 | 1 0 1 | 1 2 1 | Bc. |
| 8552 | 3 3 0 | 3 4 1 | 1 1 0 | 2 1 1 | $Bd_1$, $Bd_2$. |
| 8543 | 3 2 1 | 3 3 2 | 1 1 0 | 2 1 1 | $Be_1$, $Be_2$. |
| | | 4 3 1 | 0 1 1 | 1 1 2 | Bf. |
| 7742 | 4 1 -1 | 5 1 1 | 2 0 1 | 2 3 1 | Bg. |
| $\kappa=3$ add 2, 1 | | 5 2 0 | 1 1 1 | 2 2 2 | Same as Bg A:B.† |
| 1, 1, 1 | | | | | |
| 7652 | 3 2 -1 | 3 3 1 | 2 1 0 | 3 2 1 | $Bh_1$, $Bh_2$. |
| | | 4 3 0 | 1 1 1 | 2 2 2 | Bi. |
| 7643 | 3 1 0 | 5 1 1 | 1 0 2 | 1 3 2 | Bj. |
| | | 3 3 1 | 1 2 0 | 3 1 2 | $Bk_1$, $Bk_2$. |
| | | 4 2 1 | 1 1 1 | 2 2 2 | Bl. |
| 7544 | 2 1 1 | 3 3 1 | 0 2 1 | 2 1 3 | Bm. |
| | | 3 2 2 | 1 1 1 | 2 2 2 | Bn. |
| 6653 | 2 1 -1 | 3 1 2 | 3 0 1 | 3 4 1 | Bo. |
| $\kappa=4$ add | | 3 3 0 | 1 2 1 | 3 2 3 | Same as Bo A:B. |
| 3, 1 | | 3 2 1 | 2 1 1 | 3 3 2 | Bp. |
| 2, 1, 1 | | | | | |
| 2, 2 | | | | | |
| 6554 | 1 1 0 | 1 2 3 | 3 1 0 | 4 3 1 | Br. |
| | | 3 2 1 | 1 1 2 | 2 3 3 | Bs. |
| | | 4 1 1 | 1 0 3 | 1 4 3 | Bq. |

* $\Sigma B = \beta - AB = BC + BD$, etc. † A:B signifies that A and B are to be interchanged.

TABLE IV.—*Clutches for $p=5$.*

| Partition. | Add | $\beta-\kappa$ $\gamma-\kappa$ $\delta-\kappa$ $\epsilon-\kappa$ | (ΣB) (ΣC) (ΣD) (ΣE) | (AB) (AC) (AD) (AE) | (BC) (BD) (BE) | (CD) (CE) (DE) | No. |
|---|---|---|---|---|---|---|---|
| $7^2 2^2$ | 0222 | 4 -1 -1 -1 | 3 -1 -1 -1 | 4 1 1 1 | 1 1 1 | 0 0 0 | (1) V. |
| $\kappa=3$ add | 2211 | | 1 1 2 2 | 6 1 0 0 | 1 0 0 | 0 0 2 | (2) I. |
| Parti'n of $2\kappa$ | | | | | 0 1 0 | 0 1 1 | (3) Ca. |
| 222 | | | | | 0 0 1 | 1 0 1 | (4) Same as (3) D:E. |
| 2211 | 1221 | | 2 1 1 2 | 5 1 1 0 | 0 0 2 | 1 0 0 | (5) I. |
| | | | | | 1 0 1 | 0 0 1 | (6) $Cb_1$, $Cb_2$, $Cb_3$. |
| | | | | | 0 1 1 | 0 1 0 | (7) Same as (6) C:D. |
| * | * | * | * | * | * | * | * * * |
| $6^2 4^2$ | 2033 | 2 0 -2 -2 | 2 4 1 1 | 4 0 1 1 | 2 0 0 | 1 1 0 | (1) IV. |
| $\kappa=4$ add | 3023 | | 1 4 2 1 | 5 0 0 1 | 1 0 0 | 2 1 0 | (2) I. |
| 332 | 0323 | | 4 1 2 1 | 2 3 0 1 | 1 2 1 | 0 0 0 | (3) I. |
| 3311 | 0233 | | 4 2 1 1 | 2 2 1 1 | 2 1 1 | 0 0 0 | (4) IV. |
| 3221 | 1133 | | 3 3 1 1 | 3 1 1 1 | 3 0 0 | 0 0 1 | (5) I. |
| 2222 | | | | | 2 1 0 | 0 1 0 | (6) $C^2c_1C^2c_2C^2c_3C_2c_4$. |
| | | | | | 2 0 1 | 1 0 0 | (7) $\equiv$ (6) D:E. |
| | 3122 | | 1 3 2 2 | 5 1 0 0 | 0 0 1 | 2 1 0 | (8) I. |
| | | | | | 0 1 0 | 1 2 0 | (9) I. |
| | | | | | 1 0 0 | 1 1 1 | (10) I. |
| | 1322 | | 3 1 2 2 | 3 3 0 0 | 0 1 2 | 1 0 0 | (11) I. |
| | | | | | 0 2 1 | 0 1 0 | (12) I. |
| | | | | | 1 1 1 | 0 0 1 | (13) I. |
| | 1223 | | 3 2 2 1 | 3 2 0 1 | 2 1 0 | 0 0 1 | (14) IV. |
| | | | | | 1 2 0 | 0 1 0 | (15) I. |
| | | | | | 1 1 1 | 1 0 0 | (16) $\equiv$ (7) A:B. |
| | 2123 | | 2 3 2 1 | 4 1 0 1 | 2 0 0 | 1 0 1 | (17) IV. |
| | | | | | 1 0 1 | 2 0 0 | (18) I. |
| | | | | | 1 1 0 | 1 1 0 | (19) $C^2d_1$, $C^2d_2$. |
| | 2222 | | | 4 2 0 0 | | | (20) IV. |
| * | * | * | * | * | * | * | * * * |

TABLE IV.—*Clutches for p=5 (continued).*

| Partition. | Add | $\beta-\kappa$ | $\gamma-\kappa$ | $\delta-\kappa$ | $\epsilon-\kappa$ | $\Sigma B$ | $\Sigma C$ | $\Sigma D$ | $\Sigma E$ | $AB$ | $AC$ | $AD$ | $AE$ | $BC$ | $BD$ | $BE$ | $CD$ | $CE$ | $DE$ | No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $5^3 32$ | 0244 | 0 | 0 | −2 | −3 | 5 | 3 | 1 | 1 | 0 | 2 | 2 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | (1) $C^3y$. |
| $\kappa=5$ add | 0424 | | | | | 5 | 1 | 3 | 1 | 0 | 4 | 0 | 1 | 1 | 3 | 1 | 0 | 0 | 0 | (2) I. |
| 442 | 1144 | | | | | 4 | 4 | 1 | 1 | 1 | 1 | 2 | 1 | 4 | 0 | 0 | 0 | 0 | 1 | (3) I ABCA. |
| 4411 | | | | | | | | | | | | | | 3 | 1 | 0 | 0 | 1 | 0 | (4) VI, §12, V. |
| 433 | | | | | | | | | | | | | | 3 | 0 | 1 | 1 | 0 | 0 | (5) ≡ (4) B:C. |
| 4321 | 0433 | | | | | 5 | 1 | 2 | 2 | 0 | 4 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 0 | (6) I |
| 4222 | 0334 | | | | | 5 | 2 | 2 | 1 | 0 | 3 | 1 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | (7) ≡ (1) A:B. |
| 3331 | 0343 | | | | | 5 | 2 | 1 | 2 | 0 | 3 | 2 | 0 | 2 | 1 | 2 | 0 | 0 | 0 | (8) I. |
| 3322 | 1234 | | | | | 4 | 3 | 2 | 1 | 1 | 2 | 1 | 1 | 3 | 1 | 0 | 0 | 0 | 1 | (9) VI, §12, V. |
| | | | | | | | | | | | | | | 2 | 2 | 0 | 0 | 1 | 0 | (10) $C^2z_1$, $C^2z_2$, $C^2z_3$. |
| | | | | | | | | | | | | | | 2 | 1 | 1 | 1 | 0 | 0 | (11) VI, V. |
| | 1243 | | | | | 4 | 3 | 1 | 2 | 1 | 2 | 2 | 0 | 3 | 0 | 1 | 0 | 0 | 1 | (12) $C^4a$ |
| | | | | | | | | | | | | | | 2 | 1 | 1 | 0 | 1 | 0 | (13) ≡ 10) A:B. |
| | | | | | | | | | | | | | | 2 | 0 | 2 | 1 | 0 | 0 | (14) I. |
| | 1324 | | | | | 4 | 2 | 3 | 1 | 1 | 3 | 0 | 1 | 2 | 2 | 0 | 0 | 0 | 1 | (15) ≡ (12) A:B. |
| | | | | | | | | | | | | | | 1 | 3 | 0 | 0 | 1 | 0 | (16) I. |
| | | | | | | | | | | | | | | 1 | 2 | 1 | 1 | 0 | 0 | (17) ≡ (5) A:B. |
| | 1423 | | | | | 4 | 1 | 3 | 2 | 1 | 4 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 1 | (18) ≡ (3) A:B. |
| | | | | | | | | | | | | | | 0 | 3 | 1 | 0 | 1 | 0 | (19) I. |
| | | | | | | | | | | | | | | 0 | 2 | 2 | 1 | 0 | 0 | (20) I. |
| | 2224 | | | | | 3 | 3 | 3 | 1 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | (21) ≡ (10) A:B & B:C |
| | | | | | | | | | | | | | | 1 | 2 | 0 | 1 | 1 | 0 | (22) ≡ (21) B:C. |
| | | | | | | | | | | | | | | 2 | 1 | 0 | 1 | 0 | 1 | (23) $C^4b$. |
| | 1333 | | | | | 4 | 2 | 2 | 2 | 1 | 3 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | (24) ≡ (9) A:B. |
| | | | | | | | | | | | | | | 1 | 2 | 1 | 0 | 1 | 0 | (25) ≡ (4) A:B. |
| | | | | | | | | | | | | | | 1 | 1 | 2 | 1 | 0 | 0 | (26) I. |
| | 2233 | | | | | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | (27) I. |
| | | | | | | | | | | | | | | 2 | 1 | 0 | 0 | 1 | 1 | (28) ≡ (23) B:C A:B. |
| | | | | | | | | | | | | | | 2 | 0 | 1 | 1 | 0 | 1 | (29) ≡ (23) A:B. |
| | | | | | | | | | | | | | | 1 | 2 | 0 | 0 | 2 | 0 | (30) I. |
| | | | | | | | | | | | | | | 1 | 0 | 2 | 2 | 0 | 0 | (31) I. |
| | | | | | | | | | | | | | | 1 | 1 | 1 | 1 | 1 | 0 | (32) ≡ (11) B:C & A:B |
| | 2323 | | | | | 3 | 2 | 3 | 2 | 2 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | (33) I. |
| | | | | | | | | | | | | | | 1 | 2 | 0 | 0 | 1 | 1 | (34) ≡ (12) B:A & B:C |
| | | | | | | | | | | | | | | 0 | 2 | 1 | 1 | 1 | 0 | (35) ≡ (1) A:B & B:C |
| | | | | | | | | | | | | | | 1 | 1 | 1 | 1 | 0 | 1 | (36) ≡ (9) A:B & B:C |
| | | | | | | | | | | | | | | 2 | 1 | 0 | 0 | 0 | 2 | (37) I. |
| | | | | | | | | | | | | | | 0 | 1 | 2 | 2 | 0 | 0 | (38) I. |
| * | * | | | * | | | * | | | | * | | | | * | | | * | | * * * |

TABLE V.—*Clutches for p=6.*

| Partition. | Add | $\beta-\kappa$ | $\gamma-\kappa$ | $\delta-\kappa$ | $\epsilon-\kappa$ | $\phi-\kappa$ | $\Sigma B$ | $\Sigma C$ | $\Sigma D$ | $\Sigma E$ | $\Sigma F$ | $AB$ | $AC$ | $AD$ | $AE$ | $AF$ | $BC$ | $BD$ | $BE$ | $BF$ | $CD$ | $CE$ | $CF$ | $DE$ | $DF$ | $EF$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| * | * | | | * | | | | | * | | | | | * | | | | * | | | | * | | | * | | * * * |
| $53^5$ | 22344 | −2 | −2 | −2 | −2 | −2 | 3 | 3 | 2 | 1 | 1 | 0 | 0 | 1 | 2 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | (1) I. |
| $\kappa=5$ | | | | | | | | | | | | | | | | | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | (2) VI. |
| | | | | | | | | | | | | | | | | | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | (3) Knot D $s_2$ of $54^2 32^2$. |
| | 23334 | | | | | | 3 | 2 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | (4) VI. |
| | | | | | | | | | | | | | | | | | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | (5) VII. |
| | | | | | | | | | | | | | | | | | 0 | 2 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | (6) Knot $D_u$, of $643^2 2^2$, knot $D^2x_4$ of $54^2 32^2$ |
| | 33333 | | | | | | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | (7) I. |
| | | | | | | | | | | | | | | | | | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | (8) Knot $D^2o$ Amph. |
| * | * | | | * | | | | | * | | | | | * | | | | * | | | | * | | | * | | * * * |

TABLE VI.—*Knots of Class* VI.

| Knot. | No. | Forms. |
|---|---|---|
| I | 6 | $Da_1$, $Da_2$, $Da_3$, $Da_4$, $Da_5$, $Da_6$. |
| II | 1 | $Db$. |
| III | 4 | $Dc_1$, $Dc_2$, $Dc_3$, $Dc_4$. |
| IV | 4 | $De_1$, $De_2$, $De_3$, $De_4$. |
| V | 2 | $Df_1$, $Df_2$. |
| VI | 4 | $Dk_1$ Amph, $Dk_2$‖,* $D^2j$ Amph. |
| VII | 1 | $Dl$ Amph. |
| VIII | 3 | $Dm$, $D^2t_1$, $D^2t_2$. |
| IX | 16 | $Dn_1$ Amph, $Dn_2$‖, $Dn_3$‖, $Dn_4$‖, $D^2i_1$‖, $D^2i_2$ Amph, $D^2h_1$‖, $D^2h_2$‖, $D^2h_3$ Amph, $D^2s$ Amph. |
| X | 12 | $Du_1$, $Du_2$, $Du_3$, $Du_4$, $Du_5$, $D^2x_1$, $D^2x_2$, $D^2x_3$, $D^2x_4$, $D^2x_5$, $Do_1$, $Do_2$.† |
| XI | 12 | $Dp_1$, $Dp_2$, $Dp_3$, $Dp_4$, $Dp_5$, $Dp_6$, $D^2e_1$, $D^2e_2$, $D^2e_3$, $D^2e_4$, $D^2e_5$, $D^2e_6$. |
| XII | 9 | $Dq_1$, $Dq_2$, $Dq_3$, $D^2f_1$, $D^2f_2$, $D^2f_3$, $D^2c$, $D^2u$, $D^2h$. |
| XIII | 6 | $Dr_1$, $Dr_2$, $Dr_3$, $D^2q_1$, $D^2q_2$, $D^2q_3$. |
| XIV | 3 | $Ds$, $D^2c_1$, $D^2c_2$. |
| XV | 2 | $Dt$, $D^2d$. |
| XVI | 9 | $Dv_1$, $Dv_2$, $Dy_1$, $Dy_2$, $Dy_3$, $D^2g_1$, $D^2g_2$, $D^2y$, $D^2j$. |
| XVII | 9 | $Dw_1$‖, $Dw_2$ Amph, $Dw_3$‖, $D^2l_1$ Amph, $D^2l_2$‖, $D^2l$ Amph. |
| XVIII | 6 | $Dx_1$, $Dx_2$, $Dx_3$, $D^2a_1$, $D^2a_2$, $D^2a$. |
| XIX | 4 | $Dz_1$ Amph, $Dz_2$‖, $D^2b$ Amph. |
| XX | 1 | $D^2b$ Amph. |
| XXI | 3 | $D^2i_1$, $D^2i_2$, $D^2i_3$. |
| XXII | 1 | $D^2k$. |
| XXIII | 1 | $D^2m$. |
| XXIV | 1 | $D^2n$. |
| XXV | 3 | $D^2p_1$‖, $D^2p_2$ Amph. |
| XXVI | 3 | $D^2r_1$ Amph, $D^2r_2$. |
| XXVII | 3 | $D^2s_1$, $D^2s_2$, $D^2s_3$. |
| XXVIII | 2 | $Dv_1$, $Dv_2$. |
| XXIX | 2 | $D^2w_1$, $D^2w_2$. |
| XXX | 2 | $D^2z_1$, $D^2z_2$. |
| XXXI | 1 | $D^3d$ Amph. |
| XXXII | 1 | $D^3e$ Amph. |
| XXXIII | 4 | $D^3f$ Amph, $D^3f_2$‖, $D^3q$ Amph. |
| XXXIV | 1 | $D^3g$. |
| XXXV | 1 | $D^3k$. |
| XXXVI | 1 | $D^3m$ Amph. |
| XXXVII | 1 | $D^3o$ Amph. |
| XXXVIII | 1 | $D^3p$ Amph. |
| XXXIX | 1 | $D^3r$. |

* The symbol ‖ indicates that a form and its perversion are both included.

† The subordinate partition of $Do_2$, Plate V. should be $643^2 2^2$.

## IV.—The Amylolytic action of Diastase of Malt, as modified by various conditions; studied quantitatively. By R. H. Chittenden and Geo. W. Cummins, Ph.B.

The close relationship existing between the diastase of malt and the amylolytic ferment of saliva has led us to make a careful study of the conditions favorable to the action of the former, in the hope of obtaining confirmation of previous results obtained with the salivary ferment.* The widespread use, moreover, of malt extracts as therapeutic agents lends to the work in question a practical interest, which in no wise detracts from its value.

Falk† has recorded that the diastase of malt loses its amylolytic power under the influence of dilute acid, similar to the ferment of saliva; that it is made inactive by gastric juice and that the retarding influence of a dilute acid (say 0·0135 per cent.) on its amylolytic power is diminished by the presence of peptone, owing to the probable formation of a peptone-acid compound. Falk, moreover, states that the retarding action of hydrochloric acid is due to destruction of the ferment, since on neutralization of the acid, amylolytic power is not restored. Kjeldahl‡ has recorded that dilute acids in very small quantity retard the amylolytic action of diastase; if, however, smaller, minimum quantities of acid are added the amylolytic power of diastase is increased. The same investigator§ has also noticed a like accelerating action of very small quantities of acid on invertin. Basnitz‖ has found that the presence of carbonic acid invariably increases the amylolytic power of diastase. Detmer¶ has recorded the same fact and in addition, that small quantities of citric acid as well as of phosphoric and hydrochloric acid increase the diastatic power of malt. Larger quantities of these acids render the malt extract inactive. Detmer has also found that the presence of a very slight alkaline reaction diminishes the amylolytic power of the ferment. Brown and Heron** state that a malt extract neutralized with barium hydroxide has its amylolytic power somewhat weakened; thus im-

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* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

† Virchow's Archiv, vol. lxxxiv, p. 119.

‡ Jahresbericht für Thierchemie, 1879, p. 382.

§ Jahresbericht für Thierchemie, 1881, p. 449.

‖ Berichte der deutsch chem. Gesell., vol. xi, p. 1443.

¶ Zeitschrift für physiol. chemie, vol. vii, p. 2.

** Liebig's Annalen der Chemie, vol. cxcix, pp. 236–238.

plying that the ferment acts more vigorously in the naturally acid extract than in a neutral fluid. The same investigators found that making the extract faintly alkaline with sodium carbonate also diminished somewhat the activity of the ferment, while sodium hydroxide completely stopped the action of the ferment. A like result was also obtained on the addition of 0·05 per cent. salicylic acid.

Such are the recorded statements bearing on this question. Few quantitative results are given, and the influence of proteid matter, aside from its connection with dilute acid, has not been considered.

### *Method employed.*

A fresh malt extract was prepared for each series of experiments, since the fluid tends rapidly to become acid, owing to the development of schizomycetes. The extract was prepared from coarsely ground malted barley, by simply extracting it with water at 40° C. for two to three hours (5 grams barley to 100 c. c. water), then filtering, neutralizing and diluting to 500 c. c.

Owing to the great difficulty of obtaining perfectly neutral starch, that used in the present work was prepared from potatoes, thoroughly washed and dried, making a starch perfectly neutral to the most delicate test papers. The volume of each digestive mixture in the various experiments was 100 c. c., containing 1 gram of starch previously boiled with a portion of the water, a definite quantity of the malt extract and a given amount of acid, alkali, or proteid matter, except in the control, which was naturally free from the latter. In determining amylolytic power the digestive mixtures were warmed at 40° C. for thirty minutes, after which further ferment action was stopped by boiling the fluid. The extent of amylolytic action was then ascertained by determining in one-fourth of the fluid, made up to 100 c. c., the amount of reducing bodies by means of Allihn's* gravimetric method; the reducing bodies being then calculated, for the sake of convenience, to dextrose, from which in turn, was calculated the percentage of starch converted.

### *Influence of sodium carbonate on the amylolytic action of diastase.*

Previous experiments† with saliva have shown that the percentage of alkaline carbonate which absolutely or to a certain extent hinders its amylolytic action can be designated only for a definite mixture and not in a general sense, owing to variations in the amount of pro-

* Zeitschrift für Analytische Chemie, vol. xxii, p. 448. † Chittenden and Smith.

teid matter present and doubtless also in part to increase or decrease in the amount of ferment.

It became necessary, therefore, at first, to ascertain something regarding the relative amylolytic action of the malt extract, which contains some proteid matter. Three quantities of malt extract were employed, which by thirty minutes warming at 40° C. with 1 gram of starch in the manner already described, gave the following results:

| Malt extract. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 10 c. c. | 0·1192 gram. | 0·2428 gram. | 21·85 per cent. |
| 15 | 0·1489 | 0·3038 | 27·34 |
| 25 | 0·1543 | 0·3150 | 28·35 |

It is interesting to note here that, as in the case of the amylolytic ferment of saliva, there is no quantitative relation between the amount of ferment and the extent of amylolytic action; it is only when the ferment solution is greatly diluted that amylolytic action can be taken as a definite measure of the amount of ferment present. Kjeldahl* has likewise studied the influence of the quantity of diastase upon the amount of sugar formed under given conditions and he came to the conclusion that the formation of sugar was proportional to the amount of ferment only up to a certain point; beyond which, increase in the amount of ferment was not accompanied by proportional increase in the formation of sugar.

Preliminary experiments showed us that the ferment of malt is very susceptible to the action of sodium carbonate; the addition of even 0·025 gram of the alkaline carbonate to 15 c. c. of perfectly neutral malt extract, with subsequent dilution to 100 c. c. allowed no diastatic action whatever. Following are two series of experiments illustrating the action of different percentages of sodium carbonate† on the ferment under different degrees of dilution.

*a.* with 15 c. c. of the standard malt extract.

| $Na_2Co_3$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1371 gram. | 0·2794 gram. | 25.14 per cent. |
| 0·0005 per cent. | 0·1318 | 0·2684 | 24·15 |
| 0·0010 | 0·1274 | 0·2596 | 23.36 |
| 0·0020 | 0·0544 | 0·1124 | 10·11 |
| 0·0050 | 0·0197 | 0·0434 | 3·90 |
| 0·0080 | 0·0134 | 0·0312 | 2·80 |
| 0·0100 | 0·0135 | 0·0314 | 2·82 |
| 0·0125 | 0·0065 | 0·0148 | 1·33 |
| 0·0250 | 0 | | |

* Jahresbericht für Thierchemie, 1879, 381.

† The standard solutions of sodium carbonate employed in these experiments were made from the chemically pure anhydrous salt.

*b.* with 30 c. c. of the standard malt extract.

| $Na_2Co_3$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1650 gram. | 0·3372 gram. | 30·34 per cent. |
| 0·001 per cent. | 0·1578 | 0·3224 | 29·01 |
| 0·003 | 0·1465 | 0·2986 | 26·87 |
| 0·005 | 0·1147 | 0·2334 | 21·00 |
| 0·010 | 0·0380 | 0·0796 | 7·16 |
| 0·025 | 0·0238 | 0·0516 | 4·64 |
| 0·050 | 0 | | |

It is evident from these results that the amylolytic power of diastase, like that of ptyaline, is diminished in proportion as the percentage of alkaline carbonate is increased. Moreover, it would appear by comparison with results previously obtained* that diastase is far more susceptible to the action of sodium carbonate than ptyaline, and also that dilution of the malt extract does not so materially affect the retarding action of the different percentages of alkaline carbonate as in the case of saliva. Both of these results, however, may be due either to the presence of a larger amount of proteid matter in the saliva or to the presence of a larger proportion of ferment, or in fact, to both. It is noticeable in both series of experiments that the amylolytic power of the ferment after gradually diminishing appears to receive a sudden check, which in the larger amount of malt extract is produced by just double the percentage of carbonate requisite with the smaller amount of malt. In this way the effect of dilution is apparent and shows moreover that the exact influence of a given percentage of the alkaline carbonate can be designated only for a definite mixture.

### *Destructive action of sodium carbonate on diastase.*

In order to ascertain how far the retarding action of sodium carbonate is due to destruction of the ferment, the following experiment was tried. Six mixtures were made as follows:

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Malt extract | 30 c.c. | 30 c.c. | 30 c.c. | 30 c.c. | 30 c.c. | 30 c.c. |
| $Na_2Co_3$ sol. | 0 " | 1·25 " 0·1% | 2·5 " 0·1% | 2·5 " 0·5% | 5 " 0·5% | 10 " 0·5% |
| $H_2O$ | 20 " | 18·75 " | 17·5 " | 17·5 " | 15 " | 10 " |
| | 50 | 50 | 50 | 50 | 50 | 50 |
| Per cent. $Na_2Co_3$ | 0 | 0·0025 | 0·005 | 0·025 | 0·05 | 0·1 |

These were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures were added as follows:

* Chittenden and Smith.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 0·1 per cent. HCl | 0 | 0·42 c. c. | 0·85 c. c. | 4·25 c. c. | 8·5 c. c. | 16·95 c. c. |
| 0·5 " $Na_2Co_3$ } * | 10 c. c. | 9·75 | 9·5 | 7·5 | 5·0 | 0 |
| 0·1 " HCl } | 16·95 | 16·55 | 16·1 | 12·7 | 8·45 | 0 |

The six solutions were now exactly alike; neutral to test papers and contained the same amounts of diastase and sodium chloride. They had, however, been exposed to the action of the above percentages of sodium carbonate for 1 hour at 40° C. Their amylolytic power was now determined in the usual manner (action on 1 gram of starch in a total dilution of 100 c. c.) with the following results:

| No. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 1 | 0·1739 gram. | 0·3558 gram. | 32·02 per cent. |
| 2 | 0·1737 | 0·3554 | 31·97 |
| 3 | 0·1745 | 0·3570 | 32·13 |
| 4 | 0·0341 | 0·0722 | 6·49 |
| 5 | 0·0319 | 0·0678 | 6·10 |
| 6 | 0·0281 | 0·0602 | 5·41 |

No destructive action is apparent until 0·025 per cent. sodium carbonate is reached; warming the malt extract with 0·005 per cent. sodium carbonate causes no destruction whatever, while with 0·025 per cent. destruction is very great. The amount of malt extract (30 c. c.) experimented with, being the same as was used in determining the influence of alkaline carbonate on the amylolytic power of the ferment, the two series of results are directly comparable and show plainly that the retarding action of small percentages, in the present case up to 0·005 per cent., is due to simple retardation without destruction of the ferment. Beyond this point, however, as in the presence of 0·025 per cent. the greatly diminished amylolytic action is due to destruction of the ferment. Hence it would appear that in the case of the diastase of malt the destructive action of sodium carbonate is out of all proportion to its retarding action. This apparent difference, however, between diastase and the ptyaline of saliva is due, as we shall show later on, to the comparatively small amount of proteid matter in the malt extract. Saliva very greatly diluted, so that the percentage of proteid matter is reduced to a minimum, shows similar results.

*Influence of neutral peptone on the amylolytic action of diastase.*

It was demonstrated some time since,† that the presence of neutral peptone tends to increase the amylolytic action of neutral saliva.

* The two equalizing mixtures were united before being added to the main solutions.

† Chittenden and Ely, Amer. Chem. Jour., vol. iv, 107.

Langley and Eves* have confirmed this statement, although they do not believe in the theory of a direct stimulation of the ferment, advanced by one of us. We find now that neutral peptone added to a neutral solution of malt diastase, similarly increases its amylolytic action; the increase being even greater than noticed in the case of neutral saliva. Two series of experiments were tried with the following results; the peptone used being made perfectly neutral with a dilute solution of sodium carbonate.

*a.* with 15 c. c. of the standard malt extract.

| Peptone. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1140 gram. | 0·2320 gram. | 20·88 per cent. |
| 0·1 per cent. | 0·1545 | 0·3154 | 28·38 |
| 0·2 | 0·1512 | 0·3084 | 27·75 |
| 0·3 | 0·1494 | 0·3048 | 27·43 |
| 0·5 | 0·1457 | 0·2970 | 26·73 |
| 1·0 | 0·1427 | 0·2910 | 26·19 |

*b.* with 30 c. c. of the standard malt extract.

| | | | |
|---|---|---|---|
| 0 | 0·1785 gram. | 0·3654 gram. | 32·88 per cent. |
| 0·1 per cent. | 0·1847 | 0·3772 | 33·94 |
| 0·3 | 0·1912 | 0·3916 | 35·24 |

Peptone causes increased amylolytic action throughout; with 15 c. c. of malt extract, the smallest amount of peptone gives the greatest acceleration, which slowly diminishes as the percentage of peptone is increased; with 30 c. c. of malt extract, however, acceleration, which is much less than in the preceding series, increases with the increase in peptone. It is hard to find any reason for this acceleration in amylolytic action, other than a direct stimulation of the ferment.

*Influence of sodium carbonate on the amylolytic action of diastase in the presence of proteid matter.*

Proteid matter tends to prevent the retarding action of sodium carbonate on this ferment, as in the case of the salivary ferment. Thus, the addition of neutral peptone to a malt extract allows vigorous amylolytic action to take place in the presence of percentages of sodium carbonate, which alone would completely destroy the ferment. The following experiments, using 15 c. c. of the standard malt extract in each instance, illustrate the influence of peptone on the action of sodium carbonate.

* Journal of Physiology, vol. iv, No. 1.

With 0·5 per cent. neutral peptone.

| $Na_2CO_3$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1388 gram. | 0·2828 gram. | 25·45 per cent. |
| 0·001 per cent. | 0·1443 | 0·2942 | 26·47 |
| 0·002 | 0·1431 | 0·2918 | 26·27 |
| 0·003 | 0·1485 | 0·3030 | 27·27 |
| 0·004 | 0·1404 | 0·2860 | 25·74 |
| 0·005 | 0·1406 | 0·2864 | 25·77 |
| 0·010 | 0·1317 | 0·2682 | 24·13 |

It is thus seen that the presence of 0·5 per cent. of neutral peptone entirely prevents the retarding action of the several percentages of sodium carbonate, except in the last experiment of the series where slight retardation is apparent. It is to be remembered here that even 0·005 per cent. of sodium carbonate alone, almost completely stops the action of the ferment. What at first sight appears to be strange in this last series of experiments, is that the first three percentages of sodium carbonate cause a gradual increase in amylolytic action over that of the neutral fluid plus like percentage of peptone. The explanation of this, however, is quite simple. In studying the influence of neutral peptone on the action of the ferment (15 c. c. malt) it was found that the greatest acceleration of amylolytic action was obtained with the smallest percentage of peptone, and moreover that ferment action diminished in proportion as the peptone was increased. Now peptone undoubtedly prevents the action of sodium carbonate on the ferment by combining with it, forming an alkaline carbonate-proteid compound, possessed of but little retarding action; hence in the above experiment the first action of the smallest percentages of sodium carbonate is to diminish the amount of free peptone, thus causing slight acceleration; further on, however, the increased amount of alkaline-proteid body formed, counteracts the accelerating influence of the free peptone, when gradual retardation commences; finally, increase in the percentage of sodium carbonate leads to the presence of free sodium carbonate, when amylolytic action comes to a sudden standstill. This point being reached, increasing the percentage of peptone prevents the stoppage of ferment action. This is well illustrated by the following series of experiments, using larger percentages of both peptone and sodium carbonate, but 15 c. c. of the malt extract, as before.

| Neutral peptone. | $Na_2CO_3$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 1·0 per cent. | 0 | 0·2078 gram. | 0·4268 gram. | 38·41 per cent. |
| 1·0 | 0·010 per cent. | 0·1583 | 0·3234 | 29·10 |
| 1·0 | 0·025 | 0·1529 | 0·3122 | 27·09 |
| 1·0 | 0·050 | 0·1351 | 0·2754 | 24·78 |
| 1·0 | 0·100 | 0·0086 | 0·0209 | 1·88 |
| 2·0 | 0·100 | 0·1341 | 0·2730 | 24·57 |

Here, as before, the retarding action of sodium carbonate is held in check by the peptone, although there is slight retardation due to the alkaline-proteid body formed. Finally, the percentage of sodium carbonate being increased beyond the necessary proportion of peptone, there is a sudden cessation of ferment action. Increasing the amount of peptone, however, prevents this retarding action; evidently the alkaline-proteid body is without much effect on the ferment, only slowly diminishing its amylolytic power.

*Influence of acid proteids on the amylolytic action of diastase.*

Falk has noticed that peptone prevents to a certain extent, the retarding action of dilute acid on this ferment; no quantitative results, however, have been recorded, nor has any attempt been made to ascertain whether said action is due to simple retardation, or destruction of the ferment, or both. The action of acids, whether free or combined with proteid matter, on the diastase of malt is particularly important, in view of the rapid passage of the ferment into the stomach when taken in therapeutical preparations. Its ultimate fate must depend in great part upon the action of free and combined (proteid) hydrochloric acid upon it. It is, moreover, important to compare the behavior of the ferment in this respect, with the amylolytic ferment of saliva.

An aqueous extract of malt prepared as described, contains but little proteid matter; as a rule 2·0–2·5 c. c. of 0·1 per cent. hydrochloric acid are required to completely saturate the proteid matter contained in 30 c. c. of the neutral malt extract. This point was ascertained by use of the tropaeolin test for free acid as recommended by Danilewsky.* Thus, by way of illustration, in one instance 30 c. c. of carefully neutralized malt extract required the addition of 3·3 c. c. 0·1 per cent. HCl to give the tropaeolin reaction for free acid, and since nothing smaller than 0·003 per cent. free HCl can be detected by this method, it follows, making the proper deduction, that 2·3 c. c. of 0·1 per cent. HCl are required to completely saturate

* Centralbl. Med. Wiss., 1880; also description in Trans. Conn. Acad., vol. vi, p. 360.

the proteid matter in 30 c. c. of the extract, which would then contain 0·0023 per cent. combined HCl in the form of acid-proteids.*

Our first experiments were made to ascertain the influence of small percentages of combined acid on the action of the ferment, viz: the influence of such additions of dilute acid to the neutral malt extract as would completely saturate the proteid matter present, without giving any *free* acid whatever. The results plainly show that the addition of very small quantities of dilute hydrochloric acid to a neutral solution of diastase increases the amylolytic action of the ferment. Following are a few of the results obtained.

A. Malt extract, neutral to test papers, required 2·4 c. c. 0·1 per cent. HCl to completely saturate the proteid matter in 30 c. c.; the fluid was then acid to test papers but contained no free acid.

*a.* with 30 c. c. of the malt extract.

| Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1630 gram. | 0·3332 gram. | 29·98 per cent. |
| 0·0024 | 0·1749 | 0·3578 | 32·19 |

*b.* with 15 c. c. of the malt extract.

| | | | |
|---|---|---|---|
| 0 | 0·1285 gram. | 0·2516 gram. | 23·54 per cent. |
| 0·0012 | 0·1460 | 0·2976 | 26·78 |

B. Malt extract, neutral to test papers, required 3·1 c. c. 0·1 per cent. HCl to saturate the proteid matter in 30 c. c. of the extract.

*a.* with 30 c. c. of the malt extract.

| Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1595 gram. | 0·3258 gram. | 29·32 per cent. |
| 0·0031 | 0·1761 | 0·3602 | 32·41 |

*b.* with 15 c. c. of the malt extract.

| | | | |
|---|---|---|---|
| 0 | 0·1319 gram. | 0·2686 gram. | 24·17 per cent. |
| 0·00155 | 0·1599 | 0·3266 | 29·39 |

C. With 30 c. c. of malt extract; two distinct preparations.

| Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1575 gram. | 0·3214 gram. | 28·92 per cent. |
| 0·0023 | 0·1766 | 0·3612 | 32·50 |
| 0 | 0·1681 | 0·3438 | 30·94 |
| 0·0023 | 0·1770 | 0·3620 | 32·58 |

* Doubtless, however, it is not wholly as HCl-proteid, since the extract frequently contains a trace of sodium lactate.

Such a degree of uniformity in the results, makes it evident that the slight accelerating action of the acid-proteid is a constant one under the above conditions, and indeed all of our results in this direction clearly indicate such to be the case.

Although acid-proteid thus accelerates amylolytic action, it is still able under slightly different conditions to retard the action of the ferment and even cause destruction; thus by warming the amount of ferment (30 c. c. malt extract), used in the preceding experiment, at 40° C. for 1 hour with the acid necessary to saturate the proteids, in a total volume of 50 c. c., thus doubling the *percentage* of acid, amylolytic action was found on subsequent neutralization and testing with starch paste, to be very much diminished. The following results obtained with the two malt extracts used in C illustrate this point:

| | *a.* | *b.* | *c.* | *d.* |
|---|---|---|---|---|
| Per cent. HCl .. | 0 | 0·0046 | 0 | 0·0046 |
| Malt extract.... | 30 c. c. | 30 c. c. | 30 c. c. | 30 c. c. |
| 0·1 per cent. HCl | 0 | 2·3 " | 0 | 2·3 " |
| $H_2O$..... | 20 " | 17·7 " | 20 " | 17·7 " |
| | 50·0 c. c. | 50·0 c. c. | 50·0 c. c. | 50·0 c. c. |

These solutions were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures were added, after which amylolytic action was determined by adding starch paste, diluting to 100 c. c., warming at 40° C. for 30 minutes, etc., with the following results, expressed in the percentage of starch converted into sugar.

| *a.* | *b.* | *c.* | *d.* |
|---|---|---|---|
| 30·24 | 21·74 | 29·25 | 22·96 |

Hence, it is apparent that under these conditions, acid-proteids may exert a destructive action on the ferment. This destructive action takes place with considerable degree of rapidity, as is shown by the following experiment, which at the same time illustrates the accelerating action of smaller percentages of combined acid and confirms the preceding experiments.

The neutral malt extract required 2·4 c. c. 0·1 per cent. HCl to combine with the proteid matter in 30 c. c.

| | Destructive action. | | | Accelerating action. | |
|---|---|---|---|---|---|
| | *1.* | *2.* | *3.* | *4.* | *5.* |
| Malt extract..... | 30 c. c. | 30 c. c. | 30 c. c. | 30 c. c. | 30 c. c. |
| 0·1 per cent. HCl | 0 | 2·4 " | 2·4 " | 0 | 2·4 " |
| $H_2O$ ..... | 20 " | 17·6 " | 17·6 " | 70* " | 70* " |
| | 50 " | 50 " | 50 " | 100 | 100 |
| Per cent. HCl ... | 0 | 0·0048 | 0·0048 | 0 | 0·0024 |

* Containing one gram of starch.

The amylolytic action of 4 and 5 was tested directly by warming the mixtures at 40° C. for 30 minutes and then determining the amount of reducing bodies. No. 1 (the control) was warmed at 40° C. for 30 minutes, while No. 2 was warmed at the same temperature for 15 minutes and No. 3 for 30 minutes. Neutralizing and equalizing mixtures were added as follows:

| | *1.* | *2.* | *3.* |
|---|---|---|---|
| 0·1 per cent. $Na_2CO_3$... | 0 | 3·5 c. c. | 3·5 c. c. |
| 0·1 per cent. HCl..... | 2·4 c. c. | 0 | 0 |
| 0·1 per cent. $Na_2CO_3$... | 3·5 " | 0 | 0 |

All three were then mixed with one gram of starch and made up to 100 c. c. in order to determine amylolytic action; each solution was neutral and contained the same quantity of sodium chloride as well as the same amount of ferment and starch. Following are the results of all five:

| No. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 1 | 0·1630 gram. | 0·3332 gram. | 29·98 per cent. |
| 2 | 0·1253 | 0·2554 | 22·98 |
| 3 | 0·1195 | 0·2434 | 21·90 |
| 4 | 0·1628 | 0·3322 | 29·89 |
| 5 | 0·1749 | 0·3578 | 32·19 |

It is thus seen that with this amount of ferment, 0·0024 per cent. combined acid causes an acceleration in amylolytic action (Nos. 4 and 5) amounting to over 2 per cent. in the quantity of starch converted, while warming the same amount of ferment with the same amount of acid, but under a less degree of dilution, causes a destruction of the ferment amounting to 7 per cent. in the conversion of the starch; 15 minutes longer at 40° C. causes only a slightly increased destruction.

*Influence of acid-peptone.*

By increasing the amount of proteid matter, larger percentages of hydrochloric acid can be added to a malt extract without retarding the action of the ferment or even interfering with the accelerating action of the smaller percentages. As previously stated, Falk has shown that peptone prevents to a certain extent the retarding action of hydrochloric acid, but it does more than this, it causes acceleration in ferment action not only in neutral solution, as already shown, but in an acid solution likewise, provided there is an excess of peptone present, in which case the acid-peptone compound formed, causes greater acceleration than the same percentage of peptone alone would do if added to a neutral solution of the ferment. The follow-

ing experiments, which we have repeated many times, testify to the accuracy of this statement:

| Per cent. Peptone. | Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 0·2 | 0 | 0·2118 gram. | 0·4356 gram. | 39·20 per cent. |
| 0·2 | 0·0003 | 0·2168 | 0·4460 | 40·14 |
| 0·2 | 0·0005 | 0·2115 | 0·4348 | 39·13 |
| 0·2 | 0·0030 | 0·2165 | 0·4454 | 40·18 |
| 0·2 | 0·0050 | 0·2175 | 0·4478 | 40·30 |
| 0·2 | 0 | 0·1730 | 0·3540 | 31·86 |
| 0·2 | 0·0003 | 0·1726 | 0·3532 | 31·78 |
| 0·2 | 0·0005 | 0·1802 | 0·3688 | 33·17 |
| 0·2 | 0·0010 | 0·1794 | 0·3672 | 33·04 |
| 0·2 | 0·0030 | 0·1765 | 0·3610 | 32·49 |
| 0·2 | 0·0050 | 0·1775 | 0·3632 | 32·68 |

Acceleration in amylolytic action is here quite noticeable; the peptone, however, is in considerable excess. As the peptone approaches saturation, retardation commences as is shown by the following experiments; such a point being reached, however, retardation can be completely prevented by increasing the amount of peptone.

| Per cent. Peptone. | Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 0·2 | 0 | 0·1716 gram. | 0·3508 gram. | 31·57 per cent. |
| 0·2 | 0·008 | 0·1732 | 0·3424 | 30·81 |
| 0·2 | 0·010 | 0·1631 | 0·3334 | 30·00 |
| 0·2 | 0·012 | 0·1442 | 0·2940 | 26·46 |
| 0·5 | 0 | 0·1633 | 0·3338 | 30·04 |
| 0·5 | 0·012 | 0·1652 | 0·3376 | 30·38 |
| 0·5 | 0·025 | 0·1170 | 0·2384 | 21·45 |

The peptone employed in this last experiment required 11·8 c. c. of 0·1 per cent. hydrochloric acid to completely saturate 0·2 gram, consequently in the fourth experiment the peptone was more than completely saturated with acid, but the amount of free acid could have been only 0·0002 per cent. In the last experiment, however, retarding action is due wholly to acid-peptone, no free acid at all being present. Increasing the percentage of combined acid still further, the proteid matter at the same time being just saturated, causes far greater retardation in the action of the ferment.

| Per cent. Peptone. | Per cent. combined HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 0·5 | 0 | 0·1607 gram. | 0·3282 gram. | 29·53 per cent. |
| 0·5 | 0·012 | 0·1661 | 0·3394 | 30·54 |
| 0·5 | 0·015 | 0·1670 | 0·3412 | 28·70 |
| 0·5 | 0·025 | 0·1202 | 0·2448 | 22·03 |
| 0·5 | 0·035* | 0·0317 | 0 0674 | 6·06 |

* With this percentage the peptone is exactly saturated with acid.

All of these results show acceleration under the influence of small percentages of combined acid, followed, as the percentages are increased, by decided retardation, thus agreeing with the results previously obtained with the salivary ferment.

The destructive action of small percentages of acid-peptone on the ferment is not great in the presence of an excess of peptone, but as the peptone approaches saturation its destructive power is increased, and when completely saturated with acid, a moderate amount of peptone so combined will quickly and completely destroy the ferment. This is plainly shown in the following series of experiments:

| | *1.* | *2.* | *3.* | *4.* |
|---|---|---|---|---|
| Malt extract | 15 c. c. | 15 c. c. | 15 c. c. | 15 c. c. |
| Peptone sol.* | 20 | 20 | 20 | 20 |
| HCl 0·1 per cent. | 0 | 1 | 2 | 4 |
| $H_2O$ | 15 | 14 | 13 | 11 |
| | 50 | 50 | 50 | 50 |
| Peptone | 0·1 per cent. | 0·1 per cent. | 0·1 per cent. | 0·1 per cent. |
| Combined HCl | 0 | 0·002 | 0·004 | 0·008 |

These mixtures were warmed at 40° C. for 30 minutes. 20 c. c. of the peptone solution together with 15 c. c. of the malt extract required 4·0 c. c. of 0·1 per cent. hydrochloric acid to saturate the proteid matter, consequently in the above mixtures, 2 and 3 contained a large excess of uncombined peptone, while in 4 the proteid matter was just saturated.

Neutralizing and equalizing mixtures were added to each as follows:

| | *1.* | *2.* | *3.* | *4.* |
|---|---|---|---|---|
| 0·1 per cent. $Na_2CO_3$ | 0 | 1·5 c. c. | 3 c. c. | 5·9 c.c. |
| 0·1 " $Na_2CO_3$ | 5·9 c. c. | 4·4 | 2·9 | 0 |
| 0·1 " HCl | 4·0 | 3·0 | 3·0 | 0 |

The solutions were now, on being diluted to 100 c. c., in every respect equal. Their amylolytic power on being tested with starch paste was as follows:

| No. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 1 | 0·1595 gram. | 0·3258 gram. | 29·32 per cent. |
| 2 | 0·1527 | 0·3118 | 28·06 |
| 3 | 0·1239 | 0·2522 | 22·69 |
| 4 | trace. | | |

Hence, when the proteid matter present is only one-quarter saturated with acid, the acid-peptone so formed may exert some destruc-

* The peptone solution contained 0·250 gram peptone in 100 c. c. water and was made neutral to test papers; 20 c. c. therefore contained 0·050 gram peptone.

tive action on the ferment; when half saturated, destructive action is more pronounced; when wholly saturated it is, under the above conditions, complete.

Frequently, such small percentages of combined acid as the above will have no retarding effect whatever on amylolytic action, though if the ferment be warmed for half an hour with the same percentage of peptone and combined acid, its subsequent amylolytic power will be much reduced, owing to a partial destruction of the ferment. This is well illustrated by the following series of experiments:

| | A. | | | B. | | |
|---|---|---|---|---|---|---|
| | *1.* | *2.* | *3.* | *4.* | *5.* | *6.* |
| Malt extract..... | 15 c. c. | 15 c. c. | 15 c. c. | 15 c. c. | 15 c. c. | 15 c. c. |
| 0·1 per cent. HCl.. | 0 | 1 | 2 | 0 | 2 | 4 |
| Peptone sol. 0·5%.. | 10 | 10 | 10 | 20 | 20 | 20 |
| $H_2O$..... | 25 | 24 | 23 | 65* | 63* | 61* |
| | 50 | 50 | 50 | 100 | 100 | 100 |
| Combined HCl.... | 0 | 0·002% | 0·004% | 0 | 0·002% | 0·004% |
| Peptone ..... | 0·1% | 0·1 | 0·1 | 0·1% | 0·1 | 0·1 |

Nos. 4, 5 and 6 were warmed directly, with the starch, for 30 minutes at 40° C. and the reducing bodies determined. Nos. 1, 2 and 3 were warmed at 40° C. also for 30 minutes, then neutralizing and equalizing mixtures were added, the solutions diluted to 100 c. c. and warmed with 1 gram of starch for 30 minutes at 40° C. The following results show the amylolytic power of the six solutions:

| | No. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| A. | 1 | 0·1482 gram. | 0·3024 gram. | 27.21 per cent. |
| | 2 | 0·1412 | 0·2876 | 25·88 |
| | 3 | 0·1351 | 0·2754 | 24·78 |
| B. | 4 | 0·1594 | 0·3256 | 29·30 |
| | 5 | 0·1628 | 0·3324 | 29·91 |
| | 6 | 0·1605 | 0·3282 | 29·52 |

In series A, we see a gradual decrease in the amylolytic power of the solutions; this can be due to nothing but the destructive action of the acid-peptone compound, for the solutions are in every respect alike, being in the same degree of dilution and containing the same amount of sodium chloride, etc. The destruction is not great, as the peptones are in considerable excess. In series B, where the ferment is exposed to the direct action, in the presence of the starch, of the same percentage of peptone and acid there is no retardation of

* Containing 1 gram of starch.

amylolytic action whatever; presumably because of the rapid action of the ferment and the slow retarding action of the acid-peptone.

*Influence of free acid on the amylolytic action of diastase.*

As might be expected, free hydrochloric acid, even in very small quantity at once stops the amylolytic action of this ferment, quickly destroying it. It is interesting, however, to compare the action of very small percentages of free acid with results obtained in like manner with the salivary ferment. The first experiment gave the following results: the malt extract used, required per 30 c. c., 2·4 c. c. 0·1 per cent. hydrochloric acid to saturate the proteid matter.

| Per cent. combined HCl. | Per cent. free HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 0 | 0 | 0·1378 gram. | 0·2808 gram. | 25·27 per cent. |
| 0·0024 | 0 | 0·1584 | 0·3236 | 29·12 |
| 0·0024 | 0·001 | 0·1486 | 0·3032 | 27·29 |
| 0·0024 | 0·003 | 0·0805 | 0·1642 | 14·77 |

With another malt extract not so active, but containing the same percentage of acid-proteids, the following results were obtained:

| Free HCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·0780 gram. | 0·1592 gram. | 14·32 per cent. |
| 0·0003 per cent. | 0·0719 | 0·1470 | 13·23 |
| 0·0005 | 0·0631 | 0·1294 | 11·64 |
| 0·0020 | 0·0380 | 0·0796 | 7·16 |
| 0·0030 | 0·0091 | 0·0222 | 1·99 |

From these two series of results it is quite evident that a very small percentage of free hydrochloric acid will stop the amylolytic action of this ferment. The main action of the acid is that of destruction, killing the ferment very quickly. The following is a sample of several experiments tried, to ascertain how far retardation is due to destruction of the ferment.

| | *1.* | *2.* | *3.* | *4.* |
|---|---|---|---|---|
| Neutral malt extract | 30 c. c. | 30 c. c. | 30 c. c. | 30 c. c. |
| 0·1 per cent. HCl to saturate proteids | 2·2 " | 2·2 " | 2·2 " | 2·2 " |
| 0·1 per cent. HCl for free acid | 0 | 0·5 " | 1·0 " | 1·5 " |
| $H_2O$ | 17·8 " | 17·3 " | 16·8 " | 16·3 " |
| | 50 " | 50 " | 50·0 " | 50·0 " |
| Per cent. free HCl | 0 | 0·001 | 0·002 | 0·003 |

These solutions were warmed at 40° C. for 30 minutes, then neutralizing and equalizing mixtures were added and the amylolytic power determined. No. 1 converted the usual amount of starch into sugar, but No. 2 showed only a trace of amylolytic power and Nos. 3 and 4 none at all. Evidently then 0·001 per cent. of free acid had

all but completely destroyed the ferment by 30 minutes warming at 40° C.

In conclusion then, we have to notice a greater susceptibility on the part of this ferment to the action of acid-proteids and free acid than the salivary ferment. Whether this latter point constitutes any real difference, it is hard to say, since the apparent increase in amylolytic action noted in the presence of traces of free acid in the case of saliva (0·0001–0·0006 per cent. free HCl) involve such small quantities as to make the results somewhat questionable,* since such very small additions of acid might perhaps be used up by the phosphates or other salts present. But taking the evidence of the results and comparing them with results obtained in like manner with the diastase of malt, it would certainly appear that the latter is more susceptible to the action of free acid than the salivary ferment, though both are very readily destroyed by a few thousandths of one per cent. of free HCl.

In other respects, the ferment of malt behaves similarly to the ferment of saliva; both act better in a neutral than in an alkaline solution; proteid matter too, prevents the retarding action of alkaline carbonate and thus, as in the case of saliva, the action of a given percentage of sodium carbonate on diastase is dependent in part, upon the concentration of the fluid and the consequent amount of proteid matter present. Neutral peptone, moreover, exerts a direct stimulating effect on the amylolytic action of neutral diastase. Greatest amylolytic action, as in the case of saliva, is, however, observed in the presence of proteid matter partially saturated with acid, but larger percentages of acid-proteids may cause complete destruction of the ferment. The accelerating action of proteid matter is in great part due to its power of combining with both acid and alkaline carbonate, but in addition we cannot but recognize a direct stimulation of the ferment, as in the action of neutral peptone on a neutral solution of diastase.

Lastly, it is evident from these results, that diastase taken into the stomach must sooner or later be completely destroyed, by either the free acid or the large percentage of acid-proteids; but in the first stage of digestion, in the absence of free acid and under the protecting influence of proteid matter the conversion of starch into sugar may still go on, though soon destined to feel the effects of the gradually increasing percentage of combined acid.

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* See Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 370.

## V.—Influence of certain Therapeutic and Toxic Agents on the amylolytic action of Saliva. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Few attempts have been made to ascertain, experimentally, the influence of therapeutic and toxic substances on amylolytic action. Yet in view of the important part which the ferment of saliva plays in the digestive processes of the body and in view likewise of the great susceptibility of the ferment, it would seem especially desirable to obtain accurate data regarding the effects of many substances on its amylolytic power.

While many laborious investigations have, from time to time, been undertaken to ascertain the influence of some one or more substances on the metabolism of the body, the influence of the same substances on the digestive processes has apparently been very little considered, with the exception, however, of the more common alkali and alkali-earth salts. Likewise too, the possible action of many toxic substances on the digestive processes, as in chronic cases of poisoning, has with a few exceptions been almost entirely ignored; yet in both of these instances it is possible that much light might be obtained by a knowledge of the influence of individual substances upon proteolytic and amylolytic action.*

With these thoughts in mind, the present investigation was undertaken, and the results which we present here plainly show the importance of the work.

In selecting substances for study, we have chosen not only those noted for therapeutic or toxic power, but also those possessed of antiseptic or germicidal properties; our object being to see how far the unformed ferment of the saliva corresponds, in its behavior towards these bodies, with the formed or organized ferments.† More-

* An interesting table of comparisons by Wernitz shows the relative action of several therapeutic agents, on the various enzymes of physiological interest.—Brunton's Pharmacology, p. 86.

† A difference in action by the same substance upon formed and unformed ferments is, as stated by Brunton, a fact of great importance, for upon it may depend a useful application of the substance in medicine; thus creosote, which has but a slight action upon pepsine and ptyaline, will kill bacteria in a dilution of 1 to 1000, and thus this agent can be used to arrest fermentation in the stomach depending on the presence of low organisms, while the proteolytic action of the digestive ferment is but little interfered with.—Brunton, p. 87.

over, only neutral bodies could be experimented with, since the smallest quantity of either free acid or alkali would exert its own peculiar destructive action on the ferment.* In view of this fact also, we have invariably used chemically pure salts and those frequently recrystallized to be sure of the absence of deleterious impurities.

### *Method employed.*

A few preliminary experiments clearly indicated that the presence of very small percentages of foreign substances exercise a decided effect on the amylolytic action of saliva, and thus the investigation resolved itself into a study, not of the percentages requisite to *completely* hinder the power of the ferment, under given conditions, but of the relative action of small percentages on the amylolytic power of the ferment. This seemed to us the more important, since we soon found that substances which, present in comparatively large amount tended to hinder amylolytic action, would when present in small quantities actually increase the activity of the ferment. Hence, we deemed it best to use accurate quantitative methods for determination of amylolytic action; such as would indicate small variations with certainty.

The experiments were made in series, in which one digestion of each series served as a control for comparison. The volume of each digestive mixture was 100 c. c., in which was present 1 gram of perfectly neutral potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva† and a given quantity of the substance to be experimented with. The mixtures were warmed at 40° C. for 30 minutes, after which further action of the ferment was stopped by heating the solution to boiling. The extent of amylolytic action was then ascertained by determining in one-fourth of the solution, the amount of reducing substances by Allihn's‡ gravimetric method. From the amount of reduced copper thus obtained, the total amount of reducing bodies was calculated (as dextrose), from which in turn was calculated the percentage of starch converted.

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* Chittenden and Smith, Transactions Conn. Acad. Arts and Sciences, vol. vi, p. 343.

† The saliva was human, mixed saliva, freshly collected. It was prepared for use by being filtered, made exactly neutral, then diluted in the proportion of 1 : 5. Thus in each digestion there were present 2 c. c. of undiluted saliva.

‡ Zeitschrift für Analytische Chemie, Jahrgang xxii, p. 448.

### *Mercuric chloride.*

Sternberg* places mercuric chloride first in the list of germicides; its presence to the extent of 0·003 per cent. being sufficient to prevent the development of the micrococcus of pus, while 0·005 per cent. destroys the vitality of the same bacterial organism.

To our surprise the salt acts even more energetically on the unorganized ferment of the saliva, as the following results show:

| $HgCl_2$ | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·2385 gram. | 0·4920 gram. | 44·28 per cent. |
| 0·0005 per cent. | 0·1277 | 0·2500 | 22·50 |
| 0·0010 | 0·0925 | 0·1880 | 16·92 |
| 0·0020 | 0·0395 | 0·0824 | 7·41 |
| 0·0030 | 0·0060 | | |
| 0·0040 | 0 | | |

It is evident that the ferment of saliva is very susceptible to the action of this poison, and we have repeated the experiment, using still smaller percentages, with the following results:

| $HgCl_2$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1635 gram. | 0·3340 gram. | 30·06 per cent. |
| 0·0001 per cent. | 0·1610 | 0·3288 | 29·59 |
| 0·0002 | 0·1570 | 0·3204 | 28·83 |
| 0·0003 | 0·1545 | 0·3152 | 28·36 |

The smallest possible addition, therefore, of mercuric chloride diminishes the amylolytic power of saliva, in proportion to the amount of mercury salt added.

### *Mercuric bromide, mercuric iodide and mercuric cyanide.*

These salts of mercury, vigorous in their action as poisons, and the two former as germicides likewise, would be expected from analogy to act similarly to the chloride. Such we find to be the case with the bromide and iodide, but with the cyanide there is to be noticed, to a slight extent, an action which we find common to many substances, viz: increasing the amylolytic power of the ferment

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* Amer. Jour. Med. Sciences, April, 1883, p. 321.

For the action of the various salts studied in this work, on the organized ferments, see also Marcus and Pinet in Compt. Rend. Soc. de Biolog., 1882, pp. 718–724, or abstract in Jahresbericht für Thierchemie, 1882, p. 515; also Ch. Richet in Compt. Rend., vol. xcvii, pp. 1004–1006, or in Jahresbericht für Thierchemie, 1883, p. 418, and Robert Koch, Jahresbericht für Thierchemie, 1881, p. 471, N. Jalan de la Croix, Jahresbericht für Thierchemie, 1881, p. 476. Brunton's Pharmacology, p. 96.

when present in one percentage and diminishing it when the percentage is increased. On account of the insolubility of mercuric iodide and bromide, these salts were dissolved in water containing potassium iodide and sodium chloride respectively, in such proportion that the various digestive mixtures contained the same percentages of these salts as they did of the mercury salts.* Following are the results obtained:

| Mercury salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1295 gram. | 0·2636 gram. | 28·72 per cent. |
| $HgBr_2$ | | | |
| 0·0005 per cent. | 0·1150 | 0·2344 | 21·09 |
| 0·0010 | 0·0770 | 0·1572 | 14·14 |
| 0·0020 | 0·0340 | 0·0720 | 6·48 |
| $HgI_2$ | | | |
| 0·0010 | 0·1257 | 0·2560 | 23·04 |
| 0·0020 | 0·1180 | 0·2404 | 21·63 |
| $Hg(CN)_2$ | | | |
| 0·0005 | 0·1375 | 0·2800 | 25·20 |
| 0·0010 | 0·1445 | 0·2944 | 26·49 |
| 0·0020 | 0·1242 | 0·2528 | 22·75 |
| 0·0030 | 0·1252 | 0·2552 | 22·96 |
| 0 | 0·1319 | 0·2684 | 24·15 |
| $Hg(CN)_2$ | | | |
| 0·0500 | 0·1025 | 0·2084 | 18·75 |

In this series of results, it is to be noticed that mercuric bromide is the most energetic in its hindering action; 0·0005 per cent. being even more effective than 0·002 per cent. of the iodide. With the cyanide, however, the first two percentages stimulate or in some way give rise to an increased amylolytic action and even 0·050 per cent. of the salt does not retard the action of the ferment as much as 0·001 per cent. of mercuric bromide.

### *Cupric sulphate.*

With this salt the following results were obtained:

| $CuSO_4+5H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1712 gram. | 0·3500 gram. | 31·50 per cent. |
| 0·0005 per cent. | 0·1445 | 0·2944 | 26·49 |
| 0·0020 | 0·0530 | 0·1096 | 9·86 |
| 0·0100 | 0·0250 | 0·0540 | 4·86 |
| 0·0250 | 0 | | |

* Apparently, the double salts so formed act as vigorously as the mercury salt alone could do.

The hindering action of the copper salt is nearly as pronounced as that of mercuric chloride and even more so than the bromide and iodide of mercury.

*Lead acetate.*

With this salt, the smaller percentages experimented with show a slight stimulating action; but the larger percentages fail to retard the amylolytic action of the ferment as the preceding salts.

| $Pb(C_2H_3O_2)_2+3H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1630 gram. | 0·3332 gram. | 29·98 per cent. |
| 0·0003 per cent. | 0·1642 | 0·3356 | 30·20 |
| 0·0005 | 0·1635 | 0·3340 | 30·06 |
| 0·0010 | 0·1635 | 0·3340 | 30·06 |
| 0·0020 | 0·1595 | 0·3256 | 29·30 |
| 0·0050 | 0·1395 | 0·2840 | 25·56 |
| 0·0100 | 0·1402 | 0·2856 | 25·70 |

A second series of experiments, with still larger percentages of the lead salt, gave the following results:

| $Pb(C_2H_3O_2)_2+3H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1742 gram. | 0·3564 gram. | 32·07 per cent. |
| 0·05 per cent. | 0·1735 | 0·3548 | 31·93 |
| 0·10 | 0·1720 | 0·3516 | 31·64 |
| 0·30 | 0·1657 | 0·3384 | 30·45 |
| 0·50 | 0·1555 | 0·3172 | 28·54 |
| 1·00 | 0·1375 | 0·2800 | 25·20 |
| 3·00 | 0·0785 | 0·1600 | 14·40 |
| 5·00 | 0·0490 | 0·1016 | 9·14 |

Thus, the presence of even five per cent. of lead acetate fails to completely prevent amylolytic action.

*Arsenious oxide.*

Owing to the comparative insolubility of this substance in neutral fluids, small percentages only could be experimented with. With these, the following results were obtained:

| $As_2O_3$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1475 gram. | 0·3004 gram. | 27·03 per cent. |
| 0·0003 per cent. | 0·1507 | 0·3072 | 27·64 |
| 0·0005 | 0·1537 | 0·3136 | 28·22 |
| 0·0010 | 0·1475 | 0·3004 | 27·03 |
| 0·0020 | 0·1570 | 0·3204 | 28·83 |
| 0·0050 | 0·1390 | 0·2832 | 25·48 |
| 0·0900 | 0·1605 | 0·3276 | 29·48 |

Although the results obtained do not wholly accord with each other they still plainly show that arsenious acid, to the extent pres-

ent in these experiments, stimulates the amylolytic action of the ferment; a fact which might be expected, assuming that the acid combines with the proteids of the saliva, for as has been elsewhere* shown, acid-proteids when present in not too large an amount increase the amylolytic action of the salivary ferment.

Schäfer and Böhm† state that arsenious acid has no influence whatever on the conversion of starch into sugar by a glycerine extract of the pancreas. Possibly they sought only for retarding action, or it may be that the pancreatic ferment differs in this respect from the ferment of saliva.

### *Arsenic acid.*

This substance being still more acid than the preceding, might naturally be expected to diminish amylolytic action, when present in quantities which in the preceding would increase the activity of the ferment; and indeed there is to be seen in the results, a slight increase, followed by a rapid decrease of amylolytic action.

| $H_3AsO_4$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1755 gram. | 0·3588 gram. | 32·29 per cent. |
| 0·0005 per cent. | 0·1765 | 0·3608 | 32·47 |
| 0·0010 | 0·1635 | 0·3340 | 30·06 |
| 0·0030 | 0·0310 | 0·0660 | 5·94 |
| 0·0050 | 0 | | |

With 0·005 per cent. of arsenic acid present in the fluid, no reducing bodies were formed in the thirty minutes of the experiment, but the solution did become clear, showing the formation of soluble products. The same fact was observed in the presence of larger percentages of the acid; the starch solution becoming clear, after the addition of saliva, even in the presence of one per cent. of the acid, although, as before, no reducing bodies were formed.

### *Ammonium arsenate.*

With this salt the following results were obtained:

| $(NH_4)_3AsO_4$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1527 gram. | 0·3112 gram. | 28·08 per cent. |
| 0·0005 per cent | 0·1620 | 0·3308 | 29·77 |
| 0·0010 | 0·1630 | 0·3340 | 30·06 |
| 0·0050 | 0·1675 | 0·3420 | 30·78 |
| 0·0150 | 0·1745 | 0·3568 | 32·11 |
| 0·0250 | 0·1700 | 0·3476 | 31·28 |

* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

† Abstract in Jahresbericht für Thierchemie, 1872, p. 365.

In a second series, larger percentages were used with the following results:

| $(NH_4)_3AsO_4$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1712 gram. | 0·3500 gram. | 31·50 per cent. |
| 0·05 per cent. | 0·1475 | 0·3004 | 27·03 |
| 0·10 | 0·1147 | 0·2332 | 20·98 |
| 0·50 | 0·0165 | 0·0368 | 3·31 |
| 1·00 | The solution became clear but no reducing bodies were formed. | | |

With this salt, a very decided stimulation of the ferment is to be observed in the presence of small percentages, while increased amounts of the salt ultimately stop diastatic action.

*Potassium antimony tartrate.*

Two series of experiments were tried with this salt, with the following results:

| $K(SbO)C_4H_4O_6$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1540 gram. | 0·3144 gram. | 28·29 per cent. |
| 0·001 per cent. | 0·1610 | 0·3288 | 29·59 |
| 0·005 | 0·1660 | 0·3392 | 30·52 |
| 0·010 | 0·1760 | 0·3600 | 32·40 |
| 0·050 | 0·1760 | 0·3600 | 32·40 |
| 0·100 | 0·1745 | 0·3568 | 31·11 |
| 0·200 | 0·1750 | 0·3580 | 32·22 |
| 0 | 0·1545 | 0·3152 | 28·36 |
| 0·10 | 0·1850 | 0·3788 | 34·09 |
| 0·30 | 0·1640 | 0·3352 | 30·16 |
| 0·50 | 0·2565 | 0·5304 | 47·73 |
| 1·00 | 0·1570 | 0·3204 | 28·83 |
| 2·00 | 0·1232 | 0·2504 | 22·53 |
| 5·00 | 0·0470 | 0·0976 | 8·78 |

Here we have an illustration, more forcible than with any other salt, of the power possessed by many substances of both increasing and diminishing the action of the ferment. One* of us has for some time held that the addition of very small quantities of hydrochloric acid to neutral saliva tends to increase the amylolytic power of the ferment; that this takes place even when the proteids present are completely saturated with the acid, or in other words when there is present a *very small* amount of *free* acid, provided the acid-proteids are not present in too large an amount. It is well known that free hydrochloric acid, when present to the extent of a few thousandths of one per cent. completely stops the action of the ferment. Langley

* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 360.

and Eves* make this divergence of action of one and the same substance a ground for questioning the accuracy of such a view, for, say they, "since 0·0015 per cent. HCl decreases amylolytic action it seems very unlikely that 0·0005 per cent. should increase it." The action of many neutral salts here experimented with, where both stimulation and retardation are obtained, plainly show that such a double action, dependent simply on quantity is not an impossible one.

*Stannous chloride.*

With this salt very marked results were obtained as follows:

| $SnCl_2$ | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1475 gram. | 0·3004 gram. | 27·03 per cent. |
| 0·0003 per cent. | 0·1582 | 0·3232 | 29·08 |
| 0·0010 | The solution became clear, but no reduction. | | |
| 0·0050 | The starch was not at all altered in appearance. | | |

Here there is stimulation, followed by rapid and complete stopping of amylolytic action.

*Zinc sulphate.*

| $ZnSO_4+7H_2O$. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1495 gram. | 0·3048 gram. | 27·43 per cent. |
| 0·0003 per cent. | 0·1490 | 0·3040 | 27·36 |
| 0·0005 | 0·1510 | 0·3088 | 27·79 |
| 0·0010 | 0·1475 | 0·3004 | 27·03 |
| 0·0020 | 0·1440 | 0·2936 | 26·42 |
| 0·0050 | 0·1360 | 0·2772 | 24·94 |
| 0·0100 | 0·1260 | 0·2576 | 23·18 |
| 0 | 0·1375 | 0·2800 | 25·20 |
| 0·05 per cent. | 0·0775 | 0·1480 | 13·32 |
| 0·10 | 0·0650 | 0·1332 | 11·98 |
| 0·30 | 0·0450 | 0·0936 | 8·42 |
| 0·44 | 0 | | |

These two series of experiments plainly show a gradually diminished amylolytic action, as the percentage of the zinc salt is increased, until with 0·4 per cent. a complete stoppage is effected.

Kjeldahl† found a like retarding action on the addition of zinc sulphate to a malt extract.

In this connection it is interesting to note that Sternberg‡ finds zinc sulphate devoid of germicide value, even when used in the proportion of 20 per cent.

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* Journal of Physiology, vol. iv, No. I.

† Jahresbericht für Thierchemie, 1879, p. 382.

‡ Amer. Jour. Med. Sciences, April, 1883, p. 330.

### *Ferric chloride.*

With this salt we obtained the following results:

| $Fe_2Cl_6$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1740 gram. | 0·3560 gram. | 32·04 per cent. |
| 0·0005 per cent. | 0·1597 | 0·3260 | 29·34 |
| 0·0020 | 0·0437 | 0·0908 | 8·17 |
| 0·0100 | 0·0095 | 0·0236 | 2·12 |
| 0·0250 | 0 | | |

Sternberg states that tincture of ferric chloride is effective as a germicide (upon micrococcus) when present to the extent of 4 per cent. On the unformed ferment of the saliva, it is, as the results show, much more active, its hindering action being directly proportional to the percentage of iron salt present.

### *Ferrous sulphate.*

With this salt of iron quite different results were obtained; and as we wished simply to compare its action with that of the ferric salt, only very small percentages were experimented with.

| $FeSO_4+7H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1245 gram. | 0·2532 gram. | 22·78 per cent. |
| 0·0005 per cent. | 0·1037 | 0·2108 | 18·97 |
| 0·0020 | 0·1323 | 0·2692 | 24·22 |
| 0·0100 | 0·1365 | 0·2780 | 25·02 |

Here there is decided stimulation with the two larger percentages, while the smallest per cent. shows an apparent decrease of amylolytic action.

Kjeldahl* found that this salt exercised a strong hindering action on the amylolytic ferment of malt.

### *Potassium permanganate.*

Sternberg places this salt next to mercuric chloride in germicide value, it being efficacious in 0·12 per cent. With the unformed ferment of the saliva it is likewise active, although no more so than many other salts experimented with. Following are the results:

| $K_2Mn_2O_8$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1475 gram. | 0·3004 gram. | 27·03 per cent. |
| 0·005 per cent. | 0·1012 | 0·2060 | 18·54 |
| 0·025 | 0 | | |

* Jahresbericht für Thierchemie, 1879, p. 382.

*Magnesium sulphate.*

With this salt we obtained the following results:

| $MgSO_4+7H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1475 gram. | 0·3004 gram. | 27·03 per cent. |
| 0·025 per cent. | 0·1597 | 0·3260 | 29·34 |
| 0·500 | 0·0510 | 0·1056 | 9·50 |

Here, there is a slight increase of diastatic action with the smallest percentage, while 0·5 per cent. of the salt greatly retards the action of the ferment.

Pfeiffer* has likewise noticed the retarding effect of this salt on salivary digestion.

*Potassium cyanide.*

This salt, so powerful as a poison, was found to have a decided effect also on the salivary ferment, causing a rapid decrease in amylolytic action.

| KCN. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1245 gram. | 0·2532 gram. | 22·78 per cent. |
| 0·0005 per cent. | 0·1080 | 0·2200 | 19·80 |
| 0·0010 | 0·0896 | 0·1828 | 16·45 |
| 0·0030 | 0·0330 | 0·0700 | 6·30 |

With 1·0 per cent. and even with 5·0 per cent. of potassium cyanide, the starch solutions became clear on the addition of saliva, showing that the ferment was able to effect some change, although in neither case were any reducing bodies formed.

It is our intention at some future time, to study the exact nature of the products formed under such conditions. The ferment appears to be peculiarly affected; for while a very small percentage of a substance like potassium cyanide or borax will completely prevent the formation of reducing bodies, increasing the amount of substance added a hundred-fold, has no effect on the clearing up of the starch solution by the ferment. Some light may be thrown upon the nature of the ferment or its mode of action.

*Potassium ferrocyanide.*

A preliminary experiment showed that this salt was less active than the cyanide and therefore larger percentages were used, with the following results:

| $K_4Fe(CN)_6+3H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1417 gram. | 0·2884 gram. | 25·96 per cent. |
| 0·025 per cent. | 0·1497 | 0·3052 | 27·46 |
| 0·100 | 0·1375 | 0·2800 | 25·20 |
| 0·250 | 0·1025 | 0·2084 | 18·80 |

* Centralbl. Med. Wiss., 1885, p. 328, abstract.

Here, unlike the cyanide, there is stimulation of the ferment. Retardation of amylolytic action requires much larger percentages; thus 1·0 per cent. of ferrocyanide completely prevented the formation of reducing bodies, although soluble starch was apparently formed, as also in the presence of 5·0 per cent. of the salt.

*Potassium ferricyanide.*

The action of this salt is almost identical with that of the ferrocyanide.

| $K_6Fe_2(CN)_{12}$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1417 gram. | 0·2884 gram. | 25·96 per cent. |
| 0·025 per cent. | 0·1515 | 0·3088 | 27·79 |
| 0·100 | 0·1295 | 0·2636 | 23·72 |
| 0·250 | 0·0975 | 0·1984 | 17·85 |

Like the ferrocyanide, this salt in 1·0 and 5·0 per cent. solutions allows the partial conversion of starch into soluble products, but no reducing bodies are formed.

*Potassium nitrate and potassium chlorate.*

| Potassium salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1513 gram. | 0·3080 gram. | 27·72 per cent. |
| $KNO_3$. | | | |
| 0·20 per cent. | 0·1550 | 0·3164 | 28·47 |
| 0·50 | 0·1528 | 0·3108 | 27·97 |
| 1·00 | 0·1462 | 0·2976 | 26·78 |
| $KClO_3$. | | | |
| 0·20 | 0·1581 | 0·3228 | 29·05 |
| 0·50 | 0·1580 | 0·3228 | 29·05 |
| 1·00 | 0·1600 | 0·3268 | 29·41 |

With 5·0 per cent. of the salts, the following results were obtained:

| Potassium salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1672 gram. | 0·3416 gram. | 30·74 per cent. |
| $KNO_3$ (5·0 pr. ct.) | 0·1559 | 0·3176 | 28·58 |
| $KClO_3$ (5·0 pr. ct.) | 0·1351 | 0·2752 | 24·76 |

With these two salts it is very obvious that small fractions of one per cent. decidedly increase amylolytic action and that potassium chlorate is the more energetic of the two in this respect. With one per cent. of the salts, potassium chlorate still shows increased action, while the nitrate causes a decrease of amylolytic activity; in the presence of 5 per cent. of the salts, on the other hand, potassium chlorate causes the greatest decrease in ferment action.

Of these two oxidizing agents, potassium chlorate does not appear to have been hitherto experimented with, but with potassium nitrate

O. Nasse* found increased amylolytic action with human saliva in the presence of 4·0 per cent. of the salt. Possibly this difference in our results is dependent in part upon difference in the relative amount of salt and ferment.

### *Sodium tetraborate* [$Na_2B_4O_7 + 10H_2O$].

With this salt experiments were tried with quantities varying from 0·050 to 3·0 per cent. and in each instance the starch was dissolved, but no reducing bodies whatever were formed. Dumas† has previously noted a like retarding effect on the diastatic action of emulsin, diastase and other like ferments. Sternberg states that this salt is without germicide value, even though used in a saturated solution; its antiseptic power, i. e. its capacity for preventing the multiplication of bacterial organisms, is, however, considerable.

### *Potassium bromide and potassium iodide.*

These two common therapeutic agents gave the following results:

| Salt used. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1483 gram. | 0·3020 gram. | 27·18 per cent. |
| KBr. | | | |
| 0·5 per cent. | 0·1566 | 0·3192 | 28·72 |
| 3·0 | 0·1450 | 0·2956 | 26·60 |
| 5·0 | 0·1314 | 0·2668 | 23·51 |
| KI. | | | |
| 0·5 | 0·1550 | 0·3164 | 28·47 |
| 3·0 | 0·1557 | 0·3172 | 28·54 |
| 5·0 | 0·1467 | 0·2984 | 26·85 |

Both of these salts show a stimulating action which is more persistent in the case of the iodide than with the bromide; 5·0 per cent. of the bromide causes a marked diminution of amylolytic action.

### *Sodium chloride.*

Previous experiments have been tried with this salt by several investigators, notably by O. Nasse‡ and E. Pfeiffer.§ The former found that the presence of 4·0 per cent. of the salt [the only percentage experimented with] caused an increase in the ferment action of saliva [128 : 100]; the latter experimenter likewise found that the

* Pflüger's Archiv. für Physiologie, vol. xi, p. 150.

† Berichte der deutsch. Chem. Gesell., vol. v, p. 826.

‡ Pflüger's Archiv für Physiologie, vol. xi, p. 155.

§ Centralbl. med. Wiss., 1885, p. 329.

presence of the salt, in concentrations up to 2 per cent., greatly increased the amylolytic action of saliva.

Our results with different percentages are as follows:

| NaCl. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1660 gram. | 0·3392 gram. | 30·52 per cent. |
| 0·3 per cent. | 0·1765 | 0·3608 | 32·47 |
| 0·5 | 0·1750 | 0·3580 | 32·22 |
| 1·0 | 0·1715 | 0·3504 | 31·53 |
| 2·0 | 0·1715 | 0·3504 | 31·53 |
| 3·0 | 0·1770 | 0·3620 | 32·58 |
| 5·0 | 0·1630 | 0·3332 | 29·98 |

These accord with the results mentioned above and show, moreover, that with 5·0 per cent. of the salt, hindering action just commences. Increasing the amount of salt beyond this point, however, only slowly diminishes the action of the ferment; thus, in the presence of 10·0 per cent. of the salt, 22·78 per cent. of starch was converted into sugar, while without it 25·20 per cent. of starch was converted.

### *Morphine sulphate.*

With this alkaloid O. Nasse* has experimented, using, however, the acetate. He found that the presence of 0·1 per cent. of the salt caused a slight increase in the diastatic action of saliva (109 : 100). Our results with the sulphate of morphine are as follows:

| Alkaloid salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1245 gram. | 0·2532 gram. | 22·78 per cent. |
| 0·05 per cent. | 0·1415 | 0·2880 | 25·92 |
| 0·50 | 0·1605 | 0·3276 | 29·48 |
| 2·00 | 0·1428 | 0·2908 | 26·17 |

The stimulating action of the alkaloid salt up to 2·0 per cent. is very apparent.

### *Quinine sulphate.*

With the acetate of this alkaloid, Nasse found, by the use of 0·1 per cent., an increase in the starch-converting power of the saliva (115 : 100). With the sulphate we obtained the following results:

| Alkaloid salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1358 gram. | 0·2768 gram. | 24·91 per cent. |
| 0·05 per cent. | 0·1475 | 0·3004 | 27·03 |
| 0·50 | 0·1355 | 0·2760 | 24·84 |
| 2·00 | 0·0981 | 0·1996 | 17·96 |

* Pflüger's Archiv, vol. xi, p. 161.

In accord with Nasse's result we see that 0·05 per cent. increases the amylolytic action of the ferment. This we verified by an additional experiment which led to a like result, although not showing so great a difference as the preceding one; thus, while the saliva alone converted 23·72 per cent. starch into reducing bodies, the presence of 0·05 per cent. of quinine sulphate led to the conversion of 24·58 per cent. of starch. Voit, as quoted by v. Boeck,* has stated that quinine is without influence on the ferment of saliva.

### *Cinchonine sulphate.*

With this alkaloid, previous experiments have not to our knowledge been tried. Our results are as follows:

| Alkaloid salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1358 gram. | 0·2768 gram. | 24·91 per cent. |
| 0·05 per cent. | 0·1452 | 0·2960 | 26·64 |
| 0·50 | 0·1455 | 0·2964 | 26·67 |
| 2·00 | 0·1440 | 0·2936 | 26·42 |

### *Cinchonidine sulphate.*

This alkaloid, like the cinchonine, shows a steady accelerating action on the ferment.

| Alkaloid salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1358 gram. | 0·2768 gram. | 24·91 per cent. |
| 0·05 per cent. | 0·1505 | 0·3068 | 27·61 |
| 0·50 | 0·1460 | 0·2976 | 26·78 |
| 1·75 | 0·1498 | 0·3056 | 27·50 |

The cinchona group of alkaloids thus show throughout an accelerating influence on amylolytic action, most pronounced in the case of cinchonidine. These alkaloids have long been known to prevent putrefaction and to check alcoholic fermentation and Binz† has demonstrated that this antiseptic action, in the case of quinine at least, is due to the poisonous influence exerted by the latter upon the fungi which are the immediate cause of the putrefactive changes. Couzen, moreover, has shown that the action of cinchonine on infusoria and on fermentation is similar to that of quinine, but weaker. Hence there is no similarity of action whatever, on the two kinds of ferments.

* Zeitschrift für Biologie, vol. vii, p. 428.

† Virchow's Archiv, vol. xlvi, 1869, p. 68.

*Atropine sulphate.*

With this alkaloid we obtained the following results:

| Alkaloid salt. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1485 gram. | 0·3028 gram. | 27·25 per cent. |
| 0·025 per cent. | 0·1460 | 0·2976 | 26·78 |
| 0·050 | 0·1410 | 0·2872 | 25·62 |
| 0·200 | 0·1530 | 0·3124 | 28·11 |
| 0·500 | 0·1407 | 0·2864 | 25·77 |
| 1·000 | 0·1475 | 0·3004 | 27·03 |
| 2·000 | 0·1245 | 0·2532 | 22·78 |

The main action of the smaller percentages of this alkaloid seems to be a slightly hindering one, although there are one or two irregularities in the results which are not readily explainable. In the presence of 2·0 per cent. of atropine sulphate there is a decided diminution in amylolytic action.

In connection with this alkaloid we have to note some recent experiments of Stolnikow* of St. Petersburg. This investigator, producing artificial fever in dogs by the injection of putrid matter into the blood found, first, that the salivary and pancreatic secretions were for a time increased in amount and then rapidly diminished and finally entirely ceased. This latter action of the septic poison, Stolnikow found to be very persistent and he moreover states that in physiological action the septic poison resembles atropine. Furthermore that artificial fever, produced as described, exercises a decided influence on the *content of ferments* in the pancreatic gland; that in fevers of short duration (2–10 hours) the extract of this gland has a more energetic ferment action than the normal extract, while in fevers of long duration the corresponding extract has a much weaker action. Overlooking now the physiological explanation suggested for these facts we come to the chemical one, viz: that the septic poison possibly exerts either a destructive or hindering influence on the ferment or its action. In support of this view, Stolnikow found that large quantities of the poison did weaken the amylolytic and proteolytic action of extracts from the pancreatic gland, although small quantities of the septic ferment were without action. Likewise, Stolnikow states that small quantities of atropine sulphate are without action on a glycerine extract of the pancreas, but by adding to 10 c. c. of a glycerine extract, 5 c. c. of a 3·0 per cent. atropine sulphate solution and allowing the mixture to stand at the ordinary temperature for 10 hours, then on

* Beiträge zur Lehre von der Function des Pancreas im Fieber. Virchow's Archiv, vol. xc, p. 389, 1882.

testing the amylolytic power of the ferment its action was found to be much weaker than the control. From this fact, Stolnikow considers that the septic poison acts upon the ferment outside the body in a manner similar to atropine.

Now it is obvious, in view of the extreme susceptibility of the ferments of the saliva and pancreas to the action of acids and alkalies, that the atropine solution must be perfectly neutral. Several specimens of atropine sulphate that we have examined, have had a slight acid reaction.

In view of the apparent identity of the amylolytic ferments of the salivary and pancreatic secretions we have repeated in principle Stolnikow's experiment with human saliva, using *perfectly neutral* atropine sulphate.

To 10 c. c. of the dilute, neutral, saliva hitherto used, 0·3 gram of pure atropine sulphate was added (=3·0 per cent. of the alkaloid salt, while Stolnikow's mixture contained but 1·0 per cent.) and the solution allowed to stand for 18 hours at the Laboratory temperature. On now being added to the starch solution, diluted up to 100 c. c. [0·3 per cent. atropine sulphate] and placed at 40° C. for 30 minutes, the starch paste quickly became clear and it was found on examination that 29·16 per cent. of starch had been converted, while the control, in the presence of 0·2 per cent. of atropine sulphate, showed a conversion of 28·11 per cent. of the starch. Hence there had been no *destruction* of the salivary ferment by even 3·0 per cent. of pure atropine sulphate, although as our previous experiments show very much smaller percentages may, by their presence, *hinder the action of the ferment.*

*Strychnine sulphate and brucine sulphate.*

O. Nasse has previously studied the influence of 0·1 per cent. strychnine acetate on the diastatic action of saliva and has noted a slight increase in amylolytic action in the presence of the strychnine [109: 100]. Our results with the two alkaloids are as follows:

| Alkaloid. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·1485 gram. | 0·3028 gram. | 27·25 per cent. |
| Strychnine sulphate. | | | |
| 0·050 per cent. | 0·1444 | 0·2936 | 26·42 |
| 0·250 | 0·1448 | 0·2936 | 26·42 |
| 0·500 | 0·1462 | 0·2976 | 26·78 |
| Brucine sulphate. | | | |
| 0·050 per cent. | 0·1503 | 0·3060 | 27·54 |
| 0·500 | 0·1524 | 0·3100 | 27·90 |
| 1·000 | 0·1505 | 0·3060 | 27·54 |

With the brucine salt a slightly increased action is noticed in all three of the experiments; while with strychnine a constant diminution in amylolytic action is to be seen.

In this connection it is to be remembered, that a trace of free acid in the alkaloid salts would introduce an appreciable error into the results, and therefore all of the alkaloid salts experimented with, were especially purified for this purpose, any adhering acid being removed by repeated crystallization, etc.

The following table shows the relative acceleration and retardation of the various salts (the percentages more generally used) compared with their controls expressed as 100.

*Table showing relative amylolytic action.*

| | 0·0008 p. c. | 0·0005 p. c. | 0·001 p. c. | 0·002 p. c. | 0·005 p. c. | 0·010 p. c. | 0·025 p. c. | 0·05 p. c. | 0·1 p. c. | 0·5 p. c. | 1·0 p. c. | 2·0 p. c. | 3·0 p. c. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $HgCl_2$ | 94·3 | 50·8 | 38·2 | 16·7 | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $HgBr_2$ | ---- | 88·9 | 59·6 | 27·3 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $HgI_2$ | ---- | ---- | 97·1 | 91·2 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $Hg(CN)_2$ | ---- | 106·2 | 111·7 | 95·9 | ---- | ---- | ---- | 77·6 | ---- | ---- | ---- | ---- | ---- |
| $CuSO_4 + 5H_2O$ | ---- | 84·0 | ---- | 31·3 | ---- | 15·4 | 0 | ---- | ---- | ---- | ---- | ---- | ---- |
| $Pb(C_2H_3O_2)_2 + 3H_2O$ | 100·7 | 100·3 | 100·3 | 97·7 | 85·2 | 85·7 | --- | 99·5 | 98·6 | 88·9 | 78·5 | ---- | 28·[illegible] |
| $As_2O_3$ | 102·2 | 104·4 | 100·0 | 106·6 | 94·2 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $H_3AsO_4$ | ---- | 100·5 | 93·0 | ---- | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $(NH_4)_3AsO_4$ | ---- | 106·2 | 107·0 | ---- | 109·6 | ---- | 111·4 | 85·8 | 66·6 | 10·5 | 0 | ---- | ---- |
| $K(SbO)C_4H_4O_6$ | ---- | ---- | 104·5 | ---- | 107·9 | 114·5 | ---- | 114·5 | 120·2 | 168·3 | 101·6 | 79·4 | 30·[illegible] |
| $SnCl_2$ | 107·5 | ---- | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $ZnSO_4 + 7H_2O$ | 99·7 | 101·3 | 98·5 | 96·3 | 90·9 | 84·5 | --- | 52·8 | 47·5 | 0 | ---- | ---- | ---- |
| $Fe_2Cl_6$ | ---- | 91·5 | ---- | 25·5 | ---- | 6·61 | 0 | ---- | ---- | ---- | ---- | ---- | ---- |
| $FeSO_4 + 7H_2O$ | ---- | 83·2 | ---- | 106·3 | ---- | 109·8 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $K_2Mn_2O_8$ | ---- | ---- | ---- | ---- | 68·5 | ---- | 0 | ---- | ---- | ---- | ---- | ---- | ---- |
| $MgSO_4 + 7H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | 108·5 | ---- | ---- | 35·1 | ---- | ---- | ---- |
| $KCN$ | ---- | 86·9 | 72·2 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $K_4Fe(CN)_6 + 3H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | 105·7 | ---- | 97·0 | ---- | ---- | ---- | ---- |
| $K_6Fe_2(CN)_{12}$ | ---- | ---- | ---- | ---- | ---- | ---- | 107·0 | ---- | 91·3 | ---- | ---- | ---- | ---- |
| $KNO_3$ | ---- | ---- | ---- | ---- | ---- | --- | ---- | ---- | ---- | 100·9 | 96·6 | ---- | 92·[illegible] |
| $KClO_3$ | ---- | ---- | --- | ---- | ---- | ---- | --- | ---- | ---- | 104·8 | 106·0 | ---- | 30·[illegible] |
| $KBr$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | --- | ---- | 105·6 | ---- | ---- | 86·[illegible] |
| $KI$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 104·7 | ---- | ---- | 98·[illegible] |
| $NaCl$ | ---- | ---- | --- | ---- | ---- | ---- | ---- | ---- | ---- | 105·5 | 103·3 | 103·4 | 98·[illegible] |
| $Na_2B_4O_7 + 10H_2O$ | ---- | --- | ---- | --- | ---- | ---- | ---- | 0 | ---- | ---- | ---- | ---- | ---- |
| $(Mo)_2 . H_2SO_4 + 5H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 113·7 | ---- | 129·4 | ---- | 114·9 | ---- |
| $(Q)_2 . H_2SO_4 + 7H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 108·5 | ---- | 99·7 | ---- | 72·1 | ---- |
| $(Ci)_2 . H_2SO_4 + 2H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 106·9 | ---- | 107·0 | ---- | 106·6 | ---- |
| $(Ci\ dine)_2 . H_2SO_4 + 3H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 110·8 | ---- | 107·5 | ---- | ---- | ---- |
| $(At)_2 . H_2SO_4$ | ---- | ---- | --- | ---- | ---- | ---- | 98.2 | 94·0 | ---- | 94·5 | 99·1 | 83·5 | ---- |
| $(Sr)_2 . H_2SO_4 + 6H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 96·9 | ---- | 98·2 | ---- | ---- | ---- |
| $(Br)_2 . H_2SO_4 + H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 101·0 | ---- | 102·4 | 101·0 | ---- | ---- |

*Influence of gases on the amylolytic action of saliva.*

The well known analysis by Pflüger* of the gases of the submaxillary saliva have shown the presence of both oxygen and carbonic acid in this secretion; oxygen to the extent of 0·6 vol.-per cent. and carbonic acid, by pump extraction, 22·5 vol.-per cent. It is, moreover, a well known fact that as the saliva flows into the mouth and becomes mixed with the food during mastication much air is absorbed. Do these three gases exert any influence on the amylolytic action of the ferment with which they are so constantly in contact?

Again, the amylolytic ferment of the pancreatic secretion, so near akin, it not identical with the salivary ferment, is subjected to the influence of the reducing gases of the intestinal canal, among which hydrogen may be present to the extent of 22·0† vol.-per cent. and hydrogen sulphide in traces. What likewise is the effect of these two gases on amylolytic action?

The experiments were conducted as follows: 90 c. c. of diluted starch paste were placed in small, partially stoppered flasks and a stream of the gas allowed to pass through, until the fluid was thought to be saturated, then 10 c. c. of dilute saliva were added and the gas allowed to bubble through the solution for 30 minutes when the mixtures were boiled and the reducing bodies determined. Following are the results:

| Gases. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch. converted. |
|---|---|---|---|
| 0 | 0·1319 gram. | 0·2684 gram. | 24·15 per cent. |
| Air | 0·1365 | 0·2780 | 25·02 |
| Oxygen | 0·1511 | 0·3080 | 27·72 |
| Carbonic acid | 0·1537 | 0·3136 | 28·22 |
| Hydrogen sulphide | 0·1377 | 0·2804 | 25.23 |
| Hydrogen | 0·1248 | 0·2540 | 22·86 |

It is interesting to see that air, oxygen and carbonic acid all stimulate and approximately in proportion to the extent in which they are present in the natural secretion, while of the reducing gases hydrogen retards and hydrogen sulphide stimulates.

The following table shows the relative acceleration and retardation of the several gases, compared with the control, expressed as 100.

| | |
|---|---|
| Air | 103·6 |
| Oxygen | 114·7 |
| Carbonic acid | 116·8 |
| Hydrogen sulphide | 104.4 |
| Hydrogen | 94·6 |

* Physiologische Chemie, Hoppe-Seyler, p. 192.

† Maly in Hermann's Handbuch der Physiologie, vol. v, p. 25.

In accord with our results, Detmer* has found that the presence of carbonic acid invariably increases the amylolytic action of the diastase of malt. The same fact was previously observed by Baswitz.† O. Nasse,‡ however, has stated that the activity of ptyaline in human mixed saliva is not materially affected by oxygen, hydrogen or air. With carbonic acid, however, he noticed acceleration in amylolytic action.

*Nature of the action of the metallic and other salts.*

In what manner do the metallic and other salts act when they, by their presence, retard or completely stop the amylolytic action of saliva? Is it a process of gradual or sudden destruction of the ferment, or does the metallic salt combine with the ferment, forming a compound incapable of ferment action? or again, is the ferment mechanically thrown down with the precipitate of albumin or globulin produced by the addition of the metallic salt to saliva, or lastly does the salt by its mere presence introduce a condition unfavorable to the action of the ferment? All of these questions are interesting ones, and possibly all of them might be answered in the affirmative and be correct for some one or more of the substances experimented with.

It is obvious that the presence of 10 or 20 per cent. of such a salt as sodium chloride or potassium nitrate in a digestive mixture might retard the action of the ferment, since solutions so saturated, even with the products of digestion, do not admit of vigorous ferment action. But the larger number of metallic salts decidedly retard amylolytic action when present to the extent of only a few thousandths of one per cent., consequently their action must be of an entirely different nature. A number of these salts, such as mercuric chloride, are well known precipitants of albumin, but the saliva being so greatly diluted, in great part for this very reason, cannot yield sufficient precipitate with the mercury salt to mechanically precipitate the ferment. As a matter of fact, when the mercuric chloride solution is added to the diluted saliva, a very faint turbidity only, is produced. If now, some of the small percentages of mercuric chloride are added to the starch solution and then *larger quantities of saliva*, thus giving a larger amount of ferment together with a larger amount of accompanying albumin and globulin, what would

* Zeitschrift für physiol. Chemie, vol. vii, p. 3.

† Berichte d. deutsch. chem. Gesell., vol. xi, p. 1443.

‡ Pflüger's Archiv, vol. xv, p. 471–481.

be the effect on the amylolytic action of the ferment? Might we not expect, knowing that albumin and mercuric chloride readily combine, that the proteid matter present in the saliva, would serve as a shield to protect the ferment from the action of the mercury or other similar metallic salt? At the same time it might be supposed that, the ferment being left intact, any mercury-albumin compound formed might retard or destroy the ferment, though less energetically than the metallic salt alone.

In an attempt to throw some light upon these points the following experiments were tried:

*Action of mercuric chloride in the presence of larger amounts of ferment and proteid matter.*

| $HgCl_2$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| | *a.* with 10 c. c. of original saliva. | | |
| 0 | 0·1772 gram. | 0·3624 gram. | 32·61 per cent. |
| 0·0005 per cent. | 0·1735 | 0·3548 | 31·93 |
| 0·0010 | 0·1695 | 0·3464 | 31·16 |
| | *b.* with 5 c. c. of original saliva. | | |
| 0 | 0·1720 gram. | 0·3516 gram. | 31·64 per cent. |
| 0·0005 per cent. | 0·1340 | 0·2728 | 24·55 |

Comparing these results with those previously obtained with the same percentages of mercuric chloride, but with 2 c. c. of original saliva, we have:

| $HgCl_2$. | 2 c. c. saliva. | 5 c. c. saliva. | 10 c. c. saliva. |
|---|---|---|---|
| 0 | 44·28 per cent. | 31·64 per cent. | 32·61 per cent. |
| 0·0005 per cent. | 23·40 | 24·55 | 31·93 |
| 0·001 | 16·92 | ---- | 31·16 |

The intensity of action of the mercuric chloride, say 0·0005 per cent. in the three cases, varies greatly; thus with 2 c. c. of saliva the difference in the percentage of starch converted, between the control and the 0·0005 per cent. is 20·88, while with 5 c. c. of saliva the difference is 7·09 and with 10 c. c. of saliva only 0·68. Obviously then, the action of a given percentage of mercuric chloride can be considered as constant only for a given mixture or under definite conditions. Moreover, it would appear (in the 10 c. c.) that either the albuminous matter of the saliva has combined with all of the mercury, leaving the ferment free to act in a normal manner, except so far as it is impeded by the mercury-albumin compound, or else that only a small proportion of the ferment has been chemically precipitated, leaving an amount sufficient for energetic amylolytic action, since, as is well

known, increase or decrease in the amount of ferment is not always followed by a proportionate change in the amount of reducing bodies formed. Of these two views the former is by far the most probable. Certainly the ferment is not mechanically precipitated by the formation of a mercury-albumin precipitate; if such were the case with 10 c. c. of saliva and 0·001 per cent. of mercuric chloride, decided retardation ought to have been observed.

*Action of cupric sulphate in the presence of larger amounts of ferment and proteid matter.*

| $CuSO_4+5H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| | *a.* with 10 c. c. saliva. | | |
| 0 | 0·1830 gram. | 0·3748 gram. | 33·73 per cent. |
| 0·0005 per cent. | 0·1775 | 0·3628 | 32·65 |
| | | Difference, | 1·08 |
| | *b.* with 5 c. c. saliva. | | |
| 0 | 0·1745 gram. | 0·3568 gram. | 32·11 per cent. |
| 0·0005 per cent. | 0·1640 | 0·3352 | 30·16 |
| | | Difference, | 1·95 |
| | *c.* with 2 c. c. saliva. | | |
| 0 | 0·1645 gram. | 0·3360 gram. | 30·24 per cent. |
| 0·0005 per cent. | 0·1140 | 0·2320 | 20·88 |
| | | Difference, | 9·36 |

*Action of zinc sulphate in the presence of larger amounts of ferment and proteid matter.*

| $ZnSO_4+7H_2O$. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| | *a.* with 10 c. c. saliva. | | |
| 0 | 0·1830 gram. | 0·3748 gram. | 33·73 per cent. |
| 0·05 per cent. | 0·1737 | 0·3552 | 31·96 |
| | | Difference, | 1·77 |
| | *b.* with 5 c. c. saliva. | | |
| 0 | 0·1745 gram. | 0·3568 gram. | 32·11 per cent. |
| 0·05 per cent. | 0·1610 | 0·3288 | 29·59 |
| | | Difference, | 2·52 |
| | *c.* with 2 c. c. saliva. | | |
| 0 | 0·1645 gram. | 0·3360 gram. | 30·24 per cent. |
| 0·05 per cent. | 0·1320 | 0·2688 | 24.19 |
| | | Difference, | 6·05 |

Glancing at the *differences* in these two series of experiments, we see that they accord with what was observed in the case of mercuric chloride, viz: that a given percentage of the metallic salt will produce a constant result only under definite conditions; increasing the proportion of albuminous matter diminishes, as in the case of the mercury salt, although not so greatly, the retarding action of the salt.

Evidently, the metallic salts do not act upon the ferment by their mere presence, for if such were the case the mere combination of the salt with the albumin present, would not so materially affect the result. If, on the other hand, they do act by combining with the ferment, forming it may be an insoluble compound or one incapable of ferment action, it is fair to presume that the combination would take place immediately upon mixing the two or very soon thereafter, and thus we should expect that the length of time the two stood in contact after the first few minutes, would have no effect on the amylolytic power of the mixture, while a gradual *destructive action* would be manifested by a gradual decrease of amylolytic power. With a view to testing this point we have tried the following experiment. Three mixtures were prepared as follows:

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| Saliva | 2 c. c. | 2 c. c. | 2 c. c. |
| $H_2O$ | 8 | 7 | 8 |
| $HgCl_2$ sol. | 0 | 1 | 2 |
| | 10 | 10 | 10 |
| Per cent $HgCl_2$ | 0 | 0·005 | 0·010 |

These were placed in a bath and warmed at 40° C. for 18 hours, after which 1 c. c. of the same mercuric chloride solution was added to A and then starch and water added to all three, making the volume in each case up to 100 c. c. The mixtures were then warmed at 40° C. for thirty minutes to test the activity of the ferment; *A* containing now 0·0005 per cent. mercuric chloride, *B* the same percentage and *C* 0·001 per cent.. In *A*, 19·8 per cent. of the starch was converted into reducing bodies, while in *B* and *C* there was no amylolytic action whatever. Thus by the previous action, for this length of time, of 0·005 per cent. mercuric chloride, the ferment was rendered incapable, on subsequent dilution, of exerting any diastatic action whatever.

Again, in a similar manner it was found that by warming the saliva for thirty minutes at 40° C. with 0·005 per cent. mercuric chloride and then adding starch paste and diluting to 100 c. c. so

that the percentage of mercuric chloride was 0·0005, only 2·0 per cent. of the starch was converted, while the same quantity of saliva, in the presence of the same amount of mercury salt (0·0005 per cent.) converted 19·69 per cent. of starch.

Working with larger amounts of saliva, the following results were obtained:

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| Saliva | 10 c. c. | 10 c. c. | 10 c. c. |
| $H_2O$ | 16 | 15 | 15 |
| $HgCl_2$ sol. | 0 | 1 | 1 |
| | 26 | 26 | 26 |
| Per cent. $HgCl_2$ | 0 | 0·002 | 0·002 |

*B* was warmed at 40° C. for 15 minutes and *C* for 30 minutes; then 1 c. c. of the mercuric chloride solution was added to *A*, and all three diluted and mixed with starch paste. The three solutions were now exactly alike; all contained the same percentage of mercury salt (0·0005 per cent.) but *B* and *C* had been previously warmed with the salt for 15 and 30 minutes respectively. *A*, converted 31·32 per cent. of the starch, *B* 29·48 per cent. and *C* 27·97 per cent. Here we have what appears to be a gradual decrease in amylolytic power, but it does not seem sufficiently pronounced to account for the action of the mercury salt. It would appear rather, in this instance, as if the mercuric chloride exercised a selective action, combining with the proteid matter of the saliva, leaving the ferment free; but the mercury-proteid compound, being apparently possessed of some destructive action, exerts its influence, and thus the gradual decrease of amylolytic power noticed in *B* and *C*.

In the previous experiments, on the other hand, where *free* mercuric chloride is present, there not being sufficient albumin to combine with all of the mercury, there is apparently destructive action.

Experiments of like nature as the preceding, tried with cupric sulphate, gave the following results:

| | *A.* | *B.* | *C.* | *D.* |
|---|---|---|---|---|
| Saliva | 2 c. c. | 2 c. c. | 2 c. c. | 2 c. c. |
| $H_2O$ | 18 | 17·8 | 17·8 | 17·8 |
| $CuSO_4$ sol. | 0 | 0·2 | 0·2 | 0·2 |
| | 20 | 20·0 | 20·0 | 20·0 |
| Per cent. $CuSO_4$ | 0 | 0·0005 | 0·0005 | 0·0005 |

*B* was warmed at 40° C. for 15 minutes, *C* for 30 minutes and *D* for 1 hour; 0·2 c. c. of the cupric sulphate solution was then added to *A* and lastly starch paste and water to 100 c. c. The amylolytic

power of the four mixtures, expressed in the percentage of starch converted, was as follows:

| *A.* | *B.* | *C.* | *D.* |
|---|---|---|---|
| 28·72 | 22·35 | 23·04 | 20·23 |

With zinc sulphate, somewhat similar results were obtained:

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| Saliva | 2 c. c. | 2 c. c. | 2 c. c. |
| $H_2O$ | 18 | 16 | 16 |
| $ZnSO_4$ sol. | 0 | 2 | 2 |
| | 20 | 20 | 20 |
| Per cent. $ZnSO_4$ | 0 | 0·05 | 0·05 |

*B* was warmed at 40° C. for 30 minutes and *C* for 1 hour; then 2 c. c. of the zinc sulphate solution were added to *A*, and all three mixed with starch paste and water to 100 c. c. Each now contained 0·01 per cent. zinc sulphate and all three were then warmed at 40° C. for 30 minutes, to determine the activity of the ferment. *A*, converted 22·24 per cent. of the starch, *B* 11·88 per cent. and *C* 10·98 per cent.

These experiments would therefore indicate, on the part of the metallic salts experimented with, a destructive action towards the ferment, though loss of amylolytic power under the conditions of the experiments might also be due to more complete precipitation of the ferment in the more concentrated solution and under longer exposure to a temperature of 40° C. At the same time it is to be noticed, that any metallic-proteid compound formed with the above salts, has a far less destructive or retarding action than the free salt. Of these, the destructive action of mercuric chloride is most pronounced.

Potassium permanganate acts, doubtless, by direct destruction of the ferment through oxidation, while many of the alkali and alkali-earth salts produce their retarding effects by simple clogging of the digestive fluid; but the fact that 0·5 per cent. of one salt, as potassium antimony tartrate, for example, increases the amount of starch converted 68 per cent., and 0·5 per cent. of another salt, as magnesium sulphate, diminishes the amount of starch converted by 65 per cent., plainly indicates that there is something in the presence of these salts, dependent upon chemical constitution, that controls the action of the ferment.

## VI.—Influence of Various Inorganic and Alkaloid Salts on the Proteolytic Action of Pepsin-hydrochloric Acid. By R. H. Chittenden and S. E. Allen.

Although many experiments have been tried to ascertain the influence of various salts on ferment action since 1870, when Liebig* recorded the statement that the fermentative power of yeast is somewhat increased by a little potassium or sodium chloride, few systematic experiments, with a large variety of salts, have been made with the ferment of the gastric juice.

Alex. Schmidt† in 1876 studied the influence of sodium chloride on the digestive action of pepsin and hydrochloric acid. Wolberg‡ in 1880 studied, with the same ferment, the action of ammonium, potassium and sodium salts of nitric, hydrochloric and sulphuric acids and also the action of several alkaloids. Wernitz§ and also Petit‖ have studied the action of several metallic salts. Still later, Pfeiffer¶ has examined the influence of several alkali and alkali-earth salts on the digestive action of pepsin as well as of other ferments. Isolated experiments with single salts have likewise been recorded; these will be noticed later on.

It is thus seen that almost all work in this direction has been done with salts of the alkali and alkali-earth metals. No systematic attempt has been made to ascertain the influence on gastric digestion of the large number of metallic salts, in common use as poisons or therapeutic agents. With the exception of a few isolated cases, no accurate data are recorded bearing on this question. Observation has led to the belief that certain metallic salts interfere with digestion in the stomach, but few quantitative results are recorded to show the truth of such a belief.

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* Ueber Gährung, Quelle der Muskelkraft und Ernährung. Separatabdruck aus den Annalen der Chemie u. Pharmacie, 1870, p. 61.

† Pflüger's Archiv, vol. xiii, p. 97. Ueber die Beziehung des Kochsalzes zu einigen thierischen Fermentationsprocessen.

‡ Pflüger's Archiv, vol. xxii, p. 291. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

§ Quoted by Brunton. Pharmacology, p. 85–86.

‖ Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

¶ Ueber den Einfluss einige Salze auf verschiedene künstliche Verdauungsvorgänge. Abstract in Centralbl. med. Wiss., 1885, p. 328.

Our aim has been, therefore, to study more particularly the comparative influence on gastric digestion of various percentages of those salts, well known as poisons or therapeutic agents, which have hitherto been overlooked or but imperfectly studied. At the same time in order to make the work more complete, we have studied somewhat, the action of the alkali salts, experimented with by other observers.

### *Method employed.*

The experiments were conducted in series, in which one of each series served as a control for comparison. The artificial gastric juice employed, was made from 0·2 per cent. hydrochloric acid and a glycerine extract of pepsin, in the proportion of 10 c. c. of the latter to 1 litre of the former. The volume of each digestive mixture was 50 c. c.; made up of 25 c. c. of the above-mentioned gastric juice and 25 c. c. of 0·2 per cent. hydrochloric acid, containing the salt to be experimented with. The material to be digested, consisted of purified and dried blood-fibrin, prepared by thorough washing with water, extraction with cold and boiling alcohol and lastly with ether. It was then ground to a coarse powder and dried at 100–110° C. 1 gram of the fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for two hours, then filtered upon weighed filters by the aid of pumps, the residue washed thoroughly with water, lastly with alcohol, and finally dried at 100–110° C. until of constant weight (48 hours). The amount of fibrin digested or dissolved, is a measure of the proteolytic action.

### *Cupric sulphate.*

With this salt two series of experiments were made; one to ascertain the influence of small quantities, the other to show the effects of larger amounts of the substance.

| $CuSO_4+5H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2854 gram. | 71·46 per cent. | 100·0 |
| 0·001 per cent. | 0·2508 | 74·92 | 104·8 |
| 0·005 | 0·2650 | 73·50 | 102·8 |
| 0·010 | 0·3067 | 69·33 | 97·0 |
| 0·025 | 0·3845 | 61·55 | 86·1 |
| 0·050 | 0·3877 | 61·23 | 85·6 |
| 0 | 0·2352 | 76·48 | 100·0 |
| 0·1 | 0·5315 | 46·85 | 61·2 |
| 0·3 | 0·7585 | 24·15 | 31·5 |
| 0·5 | 0·7976 | 20·24 | 26·4 |
| 0·8 | 0·8214 | 17·86 | 23·3 |
| 1·5 | 0·8480 | 15·20 | 19·8 |

The action of the salt is very marked; with even 0·010 per cent. there is a diminution in proteolytic action amounting to 3·0 per cent., while in the presence of 0·5 per cent. of the salt, there is retardation to the amount of nearly 75 per cent. The copper salt prevents almost entirely the swelling of the fibrin and doubtless its retarding action is due in part to this fact.

### *Lead acetate.*

In view of the frequent cases of chronic poisoning with lead salts, the influence of the acetate on gastric digestion, seems especially interesting. The results, moreover, show decided action on the part of the salt; with small fractions of a per cent. pronounced increase in proteolytic action is to be noticed, while beyond 0·5 per cent. there is sudden and almost complete cessation of ferment action. In this respect, the salt acts very differently from the copper salt, with which a more gradual diminution is observed. The two largest percentages of the lead salt prevented entirely the swelling of the fibrin.

| $Pb(C_2H_3O_2)_2+3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1936 gram. | 80·64 per cent. | 100·0 |
| 0·001 per cent. | 0·1592 | 84·08 | 104·2 |
| 0·005 | 0·1892 | 81·08 | 100·5 |
| 0·010 | 0·1781 | 82·19 | 101·9 |
| 0·025 | 0·1691 | 83·09 | 103·0 |
| 0 | 0·2140 | 78·60 | 100·0 |
| 0·1 | 0·2310 | 76·90 | 97·8 |
| 0·3 | 0·4523 | 54·77 | 69·6 |
| 0·5 | 0·7419 | 25.81 | 32·8 |
| 0·8 | 0·9779 | 2·21 | 2·8 |
| 1·5 | 0·9938 | 0·62 | 0·7 |

### *Mercuric chloride.*

This salt, which showed such a marked action on the amylolytic ferment of the saliva, causes a like diminution of proteolytic action in the case of pepsin; even with 0·001 per cent. there is retardation to the extent of over 6 per cent., calling the action of the control 100.

Petit* very erroneously states that mercuric chloride up to 0·4 per cent. does not hinder the action of pepsin.

* Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

| $HgCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3759 gram. | 62·41 per cent. | 100·0 |
| 0·001 per cent. | 0·4140 | 58·60 | 93·8 |
| 0·005 | 0·4210 | 57·90 | 92·7 |
| 0 | 0·1307 | 86·93 | 100·0 |
| 0·1 | 0·4765 | 52·35 | 60·2 |
| 0·5 | 0·9007 | 9·93 | 11·4 |
| 1·0 | 1·0495 | 0 | 0 |

M. Marle* has previously experimented with mercuric chloride and has likewise found that small quantities of the salt exercise a retarding action upon gastric digestion; that as the percentage of corrosive sublimate is increased, the retarding action is correspondingly increased, although this effect is diminished up to a certain point, by increasing the strength of the digestive mixture. Marle considers that this action of mercuric chloride does not depend upon decomposition of the ferment nor upon a contraction of the albuminous matter, but rather that the salt in an acid solution enters into a chemical combination with the proteid matter and the latter is thus rendered impervious to the digestive action of the ferment.

In support of this view we offer the fact that fibrin introduced into an acid solution of pepsin in the presence of 1 per cent. of mercuric chloride, increases in weight; in the experiment given above to the extent of 49·5 milligrams. This would clearly indicate a combination of the two. Moreover, that mercuric chloride does not act by destroying the ferment we have ample proof, as the following experiment shows:

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| $H_2O$ sol. glycerine pepsin | 5 c. c. | 5 c. c. | 5 c. c. |
| HCl (0·2 per cent.) | 20 | 20 | 0 |
| $HgCl_2$ | 0 | 0·025 gram. | 0·025 gram. |
| $H_2O$ | 0 | 0 | 20 c. c. |
| | 25 | 25 c. c. | 25 |
| Per cent. $HgCl_2$ | 0 | 0·1 | 0·1 |

These three mixtures were warmed at 40° C. for 24 hours; then to *A* was added 0·025 gram $HgCl_2$ dissolved in 25 c. c. 0·2 per cent. HCl, to *B* 25 c. c. 0·2 per cent. HCl and to *C* 25 c. c. 0·4 per cent. HCl. The three solutions were now exactly alike; in *B*, however, the ferment had been exposed to the action of 0·1 per cent. $HgCl_2$ in an acid solution for 24 hours, in *C* to the action of the same percentage of the mercury salt in an aqueous solution, while *A* served as

* Abstract in Jahresbericht für Thierchemie, 1875, p. 168.

a control. 1 gram of fibrin was added to each of the mixtures, which were then placed at 40° C. for 2 hours. *B* and *C* digested the same amount of fibrin as *A*, consequently the mercuric chloride could have exerted no destructive action whatever on the ferment.

Wassilieff,* in Hoppe-Seyler's laboratory, found by comparative experiments that mercurous chloride (calomel) has no effect on the proteolytic action of pepsin.

*Mercuric bromide, Mercuric iodide and Mercuric cyanide.*

These three salts of mercury were experimented with, only so far as to compare the action of small quantities, with the action of like quantities of mercuric chloride. In using the bromide and iodide it was necessary, on account of their insolubility, to dissolve them with the aid of an equal weight of sodium chloride, consequently these two salts of mercury were doubtless present in the digestive mixtures, in part at least, as double salts. Marle, however, found that the action of mercuric chloride with small quantities of sodium chloride was not different from that of mercuric chloride alone, and doubtless the same is true of the iodide and bromide of mercury. Following are the results we obtained:

| Mercury salt. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3590 gram. | 64·10 per cent. | 100·0 |
| $HgBr_2$ | | | |
| 0·005 per cent. | 0·3731 | 62·69 | 97·8 |
| 0·025 | 0·3980 | 60·20 | 93·9 |
| $HgI_2$ | | | |
| 0·005 | 0·3114 | 68·86 | 107·4 |
| 0·025 | 0·3904 | 60·96 | 95·1 |
| $Hg(CN)_2$ | | | |
| 0·005 | 0·3105 | 68·95 | 107·5 |
| 0·025 | 0·3985 | 60·15 | 93·8 |
| 0·100 | 0·3183 | 68·17 | 106·3 |

From these it is evident that mercuric bromide is less vigorous in its hindering action than mercuric chloride; the iodide still less so, while mercuric cyanide, in similar percentages, appears to cause an increase in proteolytic action. The iodide, likewise, in the smallest percentage experimented with, causes increased proteolytic action. None of these salts then, approach mercuric chloride in the intensity of its hindering action on gastric digestion.

* Ueber die Wirkung des Calomel auf Gährungsprozesse und das Leben von Mikroorganismen. Zeitschrift f. Physiologische Chemie, vol. vi, 113.

### *Stannous chloride.*

This salt shows marked action in retarding gastric digestion; its retarding effect increasing directly with the amount of stannous chloride added.

| $SnCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2576 gram. | 74·24 per cent. | 100·0 |
| 0·025 per cent. | 0·2728 | 72·72 | 97·5 |
| 0·1 | 0·4826 | 51·74 | 69·6 |
| 0·5 | 0·7332 | 26·68 | 35·9 |
| 1·0 | 0·8155 | 18·45 | 24·8 |
| 2·0 | 0·9010 | 9·90 | 13·3 |

### *Arsenious oxide.*

This substance might naturally be expected, in view of its well known antiseptic properties, to hinder proteolytic action, more or less. It is known to hinder putrefaction and to prevent also the fermentative action of yeast. Contrary to our expectations, however, the action of arsenious oxide, so far as it is to be seen, is an accelerating one, causing increased proteolytic action. The following results were obtained:

| $As_2O_3$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2111 gram. | 78·89 per cent. | 100·0 |
| 0·05 per cent. | 0·1872 | 81·28 | 103·0 |
| 0·1 | 0·2160 | 78·40 | 99·3 |
| 0·2 | 0·1900 | 81·00 | 102·6 |
| 0·5 | 0·1707 | 82·93 | 105·1 |

The stimulating action is slight, still it is plainly recognizable. Drs. Schäfer and Böhm* have previously studied the action of arsenious acid on the digestion of albumin by artificial gastric juice, and they came to the conclusion, using 0·02 and 0·04 gram $As_2O_3$ respectively, in 34 c. c. of fluid containing egg-albumin, that arsenious oxide is without influence on the decomposition of albumin by the gastric juice ferment. Our results, though not so large in number as theirs, would indicate a slight accelerating action.

Arsenic is known, when administered in small, repeated doses, to act as a tonic; the history of arsenic-eating, indicates that the substance has some positive tonic influence over nutrition, and Dr.

* Jahresbericht für Theirchemie, 1872, p. 363. Ueber den Einfluss des Arsens auf die Wirkung der ungeformten Fermente.

Wood* states, "there is much reason for believing that it acts largely as a direct stimulant to nutrition." The results obtained in our experiments certainly accord with this statement.

### *Arsenic acid.*

The experiments tried with arsenic acid, tend to confirm the accelerating action noticed with arsenious oxide. In the first series of experiments the following results were obtained:

| $H_3AsO_4$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2696 gram. | 73·04 per cent. | 100·0 |
| 0·2 per cent. | 0·2614 | 73·86 | 101·1 |
| 0·5 | 0·1514 | 84·86 | 116·1 |
| 2·0 | 0·2583 | 74·17 | 101·5 |
| 5·0 | 0·3915 | 60·85 | 83·3 |

The accelerating action is here so very pronounced, that a second series of experiments was undertaken by way of confirmation. These give in a general way the same results, although with 0·5 per cent. the stimulating action is not so pronounced as in the first experiment.

These two series of experiments illustrate another point, which it is well to mention here, namely: that definite percentages of any particular substance do not invariably give precisely the same result, even when compared with their respective controls. They do, however, generally point in the same direction, and although not always giving exactly the same numerical expression, they show clearly the nature and extent of the action.

| $H_3AsO_4$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2490 gram. | 75·10 per cent. | 100·0 |
| 0·2 per cent. | 0·2401 | 75·99 | 101·2 |
| 0·5 | 0·2367 | 76·33 | 101·6 |
| 1·0 | 0·2335 | 76·65 | 102·0 |
| 2·0 | 0·2622 | 73·78 | 98·2 |
| 5·0 | 0·3176 | 68·24 | 90·8 |
| 0 | 0·1493 | 85·07 | 100·0 |
| 10·0 | 0·4207 | 57·93 | 68·1 |

Plainly then, arsenic acid in small percentages does accelerate the proteolytic action of pepsin-hydrochloric acid, while in large percentages (5–10) it causes a diminution in the action of the ferment. Arsenic acid tends to make the fibrin become very gelatinous.

* Therapeutics, Materia Medica and Toxicology, p. 390.

### *Zinc sulphate.*

With this salt, no experiments appear to have been hitherto made. Our results show a decided diminution in proteolytic action, even in the presence of 0·01 per cent. of the salt, while with a few thousandths of one per cent. the figures indicate a slight accelerating action. Three distinct experiments were made as follows:

| $ZnSO_4+7H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1744 gram. | 82·56 per cent. | 100·0 |
| 0·001 per cent. | 0·1609 | 83·91 | 101·6 |
| 0·005 | 0·1617 | 83·83 | 101·5 |
| 0·010 | 0·2053 | 79·46 | 96·2 |
| 0·025 | 0·2573 | 74·27 | 89·9 |
| 3·000 | 0·8400 | 16·00 | 19·3 |
| 0 | 0·1630 | 83·20 | 100·0 |
| 0·1 | 0·4848 | 51·52 | 61·9 |
| 0·3 | 0·7133 | 28·67 | 34·4 |
| 0·5 | 0·7382 | 26·18 | 31·4 |
| 0·8 | 0·7671 | 23·29 | 27·9 |
| 1·5 | 0·8202 | 17·98 | 21·6 |
| 0 | 0·1493 | 85·07 | 100·0 |
| 1·0 | 0·7683 | 23·17 | 27·2 |

A glance at these results, shows plainly a gradual decrease in proteolytic activity.

It is to be noticed that in the presence of the larger percentages of these metallic salts, the fibrin does not swell up in the 0·2 per cent. acid.

### *Manganous chloride.*

In small fractions of one per cent. this salt gave such irregular results that it is doubtful if they can be relied upon as expressing any particular action. With 0·3 per cent. the retarding action of the manganese salt commences to be very pronounced. Following are the results:

| $MnCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action |
|---|---|---|---|
| 0 | 0·1923 gram. | 80·77 per cent. | 100·0 |
| 0·001 per cent. | 0·2022 | 79·78 | 98·7 |
| 0·010 | 0·1815 | 81·85 | 101·3 |
| 0·025 | 0·2066 | 79·34 | 98·2 |
| 0·050 | 0·1855 | 81·45 | 100·8 |
| 0 | 0·1880 | 81·20 | 100·0 |
| 0·3 | 0·3687 | 63·13 | 77·7 |
| 0·8 | 0·6438 | 35·62 | 43·8 |
| 1·5 | 0·6612 | 33·88 | 41·7 |
| 3·0 | 0·7400 | 26·00 | 32·0 |

*Ferrous sulphate and Ferric chloride.*

The salts of iron, doubtless on account of their physiological importance and their great therapeutic value, have been experimented with by several observers. It has been a prevalent opinion that iron salts tend to produce disturbances in gastric digestion. Petit,* however, states as a result of experiment, that preparations of iron, in small quantities, do not hinder the action of pepsin, but in large quantities they retard the action of the ferment, doing so according to Petit, by the hydrochloric acid of the gastric juice displacing the acid of the iron salt, thus forcing the pepsin to act with a less energetic acid. Düsterhoff,† dealing with the same question, came to the conclusion that iron salts of the organic acids, exercise the greatest retarding effect on pepsin digestion, and moreover, that ferrous salts are better adapted to the organism than ferric salts. Düsterhoff also concludes that while the retarding action of iron salts is doubtless due, in part, to the setting-free of the acid of the iron salt by the acid of the gastric juice, there is in addition a specific action of the iron preparation of an unknown nature, prejudicial to digestion. Lastly, Bubnow ‡ found that moist ferric hydroxide in small quantities (not weighed) causes a scarcely recognizable diminution in proteolytic action, while the presence of 1 per cent. of ferrous chloride and ferrous sulphate causes marked retardation, as does also an excess of ferric hydroxide. The most intense action was observed on the addition of 5 per cent. of ferrous sulphate. No quantitative results, that is, percentages of albumin digested were, however, obtained.

Our experiments were made only with crystallized ferrous sulphate and ferric chloride. It appears superfluous to try the action of ferric hydroxide, which must necessarily, if in sufficient quantity, neutralize the acid of the gastric juice and thus prevent digestion by withdrawal of the free acid.

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* Quoted by Bubnow in Zeitschrift für physiologische Chemie, vol. vii, p. 316; also abstract by Herter in Jahresbericht für Thierchemie, 1880, p. 309.

† Ueber den Einfluss von Eisenpräparaten auf die Magenverdauung. Jahresbericht für Thierchemie, 1882, p. 257.

‡ Ueber den Einfluss des Eisenoxyhydrats und der Eisenoxydulsalze auf künstliche Magenverdauung und Fäulniss mit Pancreas. Zeitschrift für Physiologische Chemie, vol. vii, p. 315.

| $FeSO_4+7H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1835 gram. | 81·65 | 100·0 |
| 0·001 per cent. | 0·1916 | 80·84 | 99·0 |
| 0·005 | 0·2241 | 77·59 | 95·0 |
| 0·010 | 0·1895 | 81·05 | 99·2 |
| 0·025 | 0·2573 | 74·27 | 90·9 |
| 0·050 | 0·2773 | 72·27 | 88·5 |
| 0 | 0·1935 | 80·65 | 100·0 |
| 0·1 | 0·3467 | 65·33 | 81·0 |
| 0·3 | 0·7274 | 27·26 | 33·8 |
| 0·8 | 0·8080 | 19·20 | 23·8 |
| 1·5 | 0·8447 | 15·53 | 19·2 |

Here, with the ferrous salt, we find pronounced diminution of proteolytic action, commencing even with 0·001 per cent. With ferric chloride, the following results were obtained.

| $Fe_2Cl_6$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1842 gram. | 81·58 per cent. | 100·0 |
| 0·001 per cent. | 0·2111 | 78·89 | 96·7 |
| 0·005 | 0·2059 | 79·41 | 97·3 |
| 0·010 | 0·2165 | 78·35 | 96·0 |
| 0·050 | 0·2332 | 76·68 | 93·9 |
| 0 | 0·1961 | 80·39 | 100·0 |
| 0·3 | 0·6526 | 34·74 | 43·2 |
| 0·5 | 0·8035 | 19·65 | 24·4 |
| 0·8 | 0·8794 | 12·06 | 15·0 |
| 3·0 | 0·9582 | 4·18 | 5·2 |

A comparison of the two series of results, shows no pronounced and constant difference in the amount of action between the two iron salts; both retard proteolytic action about equally; although with the larger amounts, as with 0·5 per cent. and beyond, ferric chloride appears the most injurious. Comparing the results with those obtained with the manganese salt, which of late has been recommended as a therapeutic agent where iron cannot be taken, we see that the manganese is throughout, far less injurious than the two salts of iron.

As to the manner in which the iron salts produce their retarding effect on proteolytic action, it is evident that it cannot be due to a simple displacement of the acid of the iron salt, by which the pepsin is made to act with a less compatible acid, since ferric chloride acts similarly to the sulphate, in which case there could be no such injurious replacement.

### *Magnesium sulphate.*

Pfeiffer* alone appears to have studied the influence of magnesium sulphate on gastric digestion. He found that retarding action commenced in the presence of 0·24 per cent. of the salt and was very great in the presence of 4·0 per cent. Our results show decided retarding action, even in the presence of 0·005 per cent. of the crystallized salt. At the same time, it is to be remembered throughout, that probably differences in the strength of gastric juice, would cause some variation in the amount of retardation, produced by any given percentage.

| $MgSO_4+7H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1081 gram. | 89·19 per cent. | 100·0 |
| 0·005 per cent. | 0·1910 | 80·90 | 90·7 |
| 0·010 | 0·2330 | 76·70 | 86·0 |
| 0·050 | 0·3260 | 67·40 | 75·5 |
| 0·100 | 0·4428 | 55·72 | 62·3 |
| 0 | 0·2605 | 73·95 | 100·0 |
| 0·3 | 0·7551 | 24·49 | 33·1 |
| 0·5 | 0·7886 | 21·14 | 28·5 |
| 0·8 | 0·8250 | 17·50 | 23 7 |
| 1·5 | 0·8891 | 11·09 | 15·0 |
| 3·0 | 0·8894 | 11·06 | 14·9 |

It is noticeable here, that while the retarding influence of the salt, becomes more and more pronounced as the percentage is increased, there comes a point (1·5 per cent.) when further addition does not materially influence the action of the ferment.

### *Potassium permanganate.*

This salt, as with the amylolytic ferment of the saliva, shows very energetic action. Its influence is, without doubt, due to rapid oxidation and consequent destruction of the ferment; indeed, the (at first) bright red color of the solution became almost immediately bleached out and the solution, at the same time, completely deprived of proteolytic power. The following results testify to its extreme activity.

| $K_2Mn_2O_8$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1951 gram. | 80·48 per cent. | 100·0 |
| 0·005 per cent. | 0·8278 | 17·22 | 21·3 |
| 0·010 | 0·9949 | 0·51 | 0·6 |

It is thus more active in preventing proteolytic action, in gastric juice of the strength used, than in hindering the development of

* Abstract in Centralbl. med. Wiss., 1885, p. 328.

bacteria; although doubtless, the action of any one percentage is dependent in part, upon the amount of organic matter present. Marcus and Pinet* found that the permanganate in 0·1 per cent. would prevent the development of bacteria and in 1·5 per cent. would kill the fully developed organisms.

### *Potassium dichromate.*

A single experiment with this salt gave the following results; showing a decided retarding action on gastric digestion.

| $K_2Cr_2O_7$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2028 gram. | 79·72 per cent. | 100·0 |
| 0·01 per cent. | 0·2476 | 75·24 | 94·4 |
| 0·10 | 0·6383 | 36·17 | 45·3 |

### *Potassium cyanide.*

Potassium cyanide we found very active in diminishing the digestive power of pepsin; due in great part doubtless, to decomposition of the cyanide by the hydrochloric acid of the gastric juice with conseqent formation of pepsin-hydrocyanic acid. Our first results were as follows:

| KCN. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3255 gram. | 67·45 per cent. | 100·0 |
| 0·25 per cent. | 0·9687 | 3·13 | 4·6 |
| 0·50 | 0·9912 | 0·88 | 1·3 |

Here, the fibrin did not swell at all, indicating the probable absence of free hydrochloric acid, although of course the potassium cyanide might, *per se*, prevent swelling.

With very much smaller percentages of cyanide, we obtained the following results:

| KCN. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3098 gram. | 69·02 per cent. | 100·0 |
| 0·005 per cent. | 0·4376 | 56·24 | 81·5 |
| 0·025 | 0·3750 | 62·50 | 90·5 |

### *Potassium ferrocyanide.*

With this salt, the results are practically the same as with potassium cyanide; almost complete stopping of proteolytic action, even in the presence of small fractions of one per cent.

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* Action de quelques substances sur les bactéries de la putréfaction, Compt. rend. Soc. de Biolog., 1882, p. 718. Abstract in Jahresbericht für Thierchemie, 1882, p. 515.

| $K_4Fe(CN)_6+3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3717 gram. | 62·83 per cent. | 100·0 |
| 0·05 per cent. | 0·5969 | 40·31 | 64·1 |
| 0·10 | 0·7922 | 20·78 | 33·0 |
| 0·25 | 0·9585 | 4·15 | 6·6 |
| 0·5 | 1·0 | 0 | 0 |
| 0 | 0·3098 | 69·02 | 100·0 |
| 0·005 | 0·3562 | 64·38 | 93·3 |
| 0·025 | 0·3471 | 65·29 | 94·5 |

*Potassium chlorate and Potassium nitrate.*

These two oxidizing agents produce almost exactly the same effect on pepsin-hydrochloric acid digestion; a retarding action directly proportional to the amount of salt present.

| $KClO_3$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2683 gram. | 73·17 per cent. | 100·0 |
| 0·3 per cent. | 0·4565 | 54·35 | 74·3 |
| 0·8 | 0·7019 | 29·81 | 40·7 |
| 1·5 | 0·8173 | 18·27 | 25·0 |
| 3·0 | 0·8707 | 12·93 | 17·6 |
| $KNO_3$. | | | |
| 0 | 0·2870 gram. | 71·30 per cent. | 100·0 |
| 0·3 per cent. | 0·5370 | 46·30 | 64·9 |
| 0·5 | 0·6247 | 37·53 | 52·6 |
| 0·8 | 0·7158 | 28·42 | 39·8 |
| 1·5 | 0·8148 | 18·52 | 25·9 |
| 3·0 | 0·8907 | 10·93 | 15·3 |

With potassium chlorate no experiments have been previously tried; with potassium nitrate, however, Wolberg* experimenting with quantities varying from 0·5 to 8·0 per cent. found in every instance, diminution in the proteolytic action of his pepsin solution. This, however, amounted to but little, except in the presence of 8 grams (8 per cent.) of the nitrate, where there was a diminution in the amount of fibrin digested, equal to 49·0 per cent. Even with 6 per cent. of the salt, Wolberg found after 24 hours, only a diminution of 6·8 per cent. in the fibrin digested.

In quantity, therefore, our results do not accord at all with Wolberg's, since as the table shows, even 0·3 per cent. of potassium nitrate caused a diminution in the quantity of fibrin digested, amounting to 35·1 per cent., when compared with the control (100); while the pres-

* Pflüger's Archiv, vol. xxii, p. 300. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

ence of 3 per cent. of the salt caused a diminution in proteolytic action amounting, in the quantity of fibrin digested, to nearly 85 per cent. The only apparent explanation of this difference in the results [unless due to difference in the amount of ferment] is in the length of time the mixtures were warmed at 40° C.; in Wolberg's 24 hours, in ours 2 hours. This, if the true reason of the difference, would imply on the part of the ferment, ability to gradually overcome the influence of small amounts of the substance and thus eventually to digest an equal quantity of proteid matter. This, however, would in turn imply that the object sought for, viz: the influence of different quantities or percentages of a substance on the action of the ferment is lost sight of. The length of time best adapted to the experiment, is naturally that which will bring out most clearly and decisively all *differences* of action.

### *Sodium tetraborate* (Borax) *and Boracic acid.*

Sternberg's experiments* with both of these substances, have shown that, although possessed of no germicide value, they prevent the multiplication of bacterial organisms and are thus valuable antiseptics.

Wolberg, in experiments made with artificial gastric juice, found that in a 24 hours digestion, 0·5 gram (0·5 per cent.) of borax caused a slight acceleration in proteolytic action (0·4 per cent.), while with 1 per cent. of the salt, retardation occurred to the extent of 23·3 per cent., and in the presence of 4·0 per cent. almost complete stopping of proteolytic action. Our results, however, fail to show any stimulating action on the part of the borate, although retardation is very pronounced.

| $Na_2B_4O_7+10H_2O$. | Undigested residue. | Fibrin digested | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3610 gram. | 63·90 per cent. | 100·0 |
| 0·05 per cent. | 0·3852 | 61·48 | 96·2 |
| 0·20 | 0·4080 | 59·20 | 92·6 |
| 0·5 | 0·7710 | 22·90 | 35·8 |
| 1·0 | 0·9899 | 1·01 | 1·5 |

Doubtless, the retarding action of this salt is due wholly to the liberation of boracic acid and the consequent neutralization of the hydrochloric acid of the gastric juice. Boracic acid itself, offers no obstacle to the proteolytic action of pepsin-hydrochloric acid; on the contrary it increases it, but pepsin-boracic acid has little digestive

* Amer. Jour. Med. Sciences, April, 1883, p. 335.

power. The influence of boracic acid on pepsin-hydrochloric acid is seen from the following experiments:

| $H_3BO_3$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0 2395 gram. | 76 05 per cent. | 100·0 |
| 0·1 per cent. | 0·2232 | 77·68 | 102·1 |
| 0 | 0·2049 | 79·51 | 100·0 |
| 0·5 | 0·1875 | 81·25 | 102·2 |
| 3·0 | 0·1729 | 82·71 | 104·2 |
| 6·0 | 0·1445 | 85·55 | 107·6 |

Evidently then, the action of borax consists simply in withdrawing from the pepsin the hydrochloric acid of the gastric juice. The low digestive power of pepsin and boracic acid is shown by the following experiment:

| | *A.* | *B.* | *C.* | *D.* |
|---|---|---|---|---|
| $H_2O$ sol. pepsin | 50 c. c. | 50 c. c. | 50 c. c. | 50 c. c. |
| HCl 0·2 per cent. | 50 | 0 | 0 | 0 |
| $H_3BO_3$ | 0 | 0·2 gram. | 0·3 gram. | 0·5 gram. |
| $H_2O$ | 0 | 50 c. c. | 50 c. c. | 50 c. c. |
| | 100 | 100 | 100 | 100 |
| | 0·1 % HCl | 0·2 % $H_3BO_3$ | 0·3 % $H_3BO_3$ | 0·5 % $H_3BO_3$ |

To each, was added 1 gram of purified fibrin, after which the mixtures were warmed at 40° C. for 2 hours. Following are the results:

| | *A.* | *B.* | *C.* | *D.* |
|---|---|---|---|---|
| Wt. of undigested residue | 0·1180 | 0·9615 | 0·9705 | 0·9620 |
| Per cent. digested | 88·20 | 3·85 | 2·95 | 3·80 |

### *Ammonium oxalate.*

With this salt the following results were obtained:

| $(NH_4)_2C_2O_4+2H_2O$. | Undigested. residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3254 gram. | 67·46 per cent. | 100·0 |
| 0·010 per cent. | 0·3579 | 64·20 | 95·1 |
| 0·025 | 0·3627 | 63·73 | 94·6 |
| 0·1 | 0·3920 | 60·80 | 90·1 |
| 0·5 | 0·9049 | 9·51 | 14·1 |
| 0 | 0·3098 | 69·02 | 100·0 |
| 1·0 | 0·9958 | 0 42 | 6·6 |

As to the cause of this retarding action, it is probable, that, as in the case of borax, the oxalic acid of the salt is displaced and the ferment compelled to act with the acid thus liberated. But if we compare the results of the present series with those of the preceding,

we find that, with like percentages of the two salts, ammonium oxalate has far the greater retarding power, and yet it is well known that the ferment acts well when combined with oxalic acid. Moreover, our experiments show further on, that ammonium chloride has no greater retarding power than sodium chloride. Hence, there must be some reason, other than the one mentioned above, to account for all of the retarding action manifested by the oxalate.

Petit* states, that the maximum digestive action of oxalic acid, with 0·2–0·4 per cent. of his pepsin, is attained with 0·5–4·0 per cent. of the acid, according to the amount of pepsin.

The comparative digestive power of pepsin-oxalic acid and pepsin-hydrochloric acid is shown by the following experiments:†

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| $H_2O$ sol. pepsin | 50 c. c. | 50 c. c. | 50 c. c. |
| 0·2 per cent. HCl | 50 | 0 | 0 |
| $C_2H_2O_4$ | 0 | 0·5 gram. | 1·0 gram. |
| $H_2O$ | 0 | 50 c. c. | 50 c. c. |
| | 100 | 100 | 100 |
| | 0·1 % HCl | 0·5 % $C_2H_2O_4$ | 1·0 % $C_2H_2O_4$ |

Warmed at 40° C. for 2 hours with 1 gram of pure, dry fibrin, the following results were obtained:

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| Wt. of undigested residue | 0·2170 | 0·4450 | 0·4371 |
| Per cent. digested | 78·30 | 55·45 | 56·29 |

A second series, with larger amounts of oxalic acid, gave the following results:

| | A. | B. | C. |
|---|---|---|---|
| $H_2O$ sol. pepsin | 50 c. c. | 50 c. c. | 50 c. c. |
| 0·2 per cent. HCl | 50 | 0 | 0 |
| $C_2H_2O_4$ | 0 | 1·5 grams. | 2·0 grams. |
| $H_2O$ | 0 | 50 c. c. | 50 c. c. |
| | 100 | 100 | 100 |
| | 0·1 per cent. HCl | 1·5 per cent. $C_2H_2O_4$ | 2·0 per cent. $C_2H_2O_4$ |
| Undigested residue | 0·1085 gram. | 0·3090 gram. | 0·3640 gram. |
| Per cent. digested | 89·15 | 69·10 | 63·60 |

These four results being placed on the same basis, i. e. compared with their respective controls (100) show as follows:

* Jahresbericht für Thierchemie, 1880, p. 309.

† Made in this laboratory by Mr. R. W. Pinney.

| Per cent. of acid. | Relative proteolytic action |
|---|---|
| 0·1 HCl | 100·0 |
| 0·5 $C_2H_2O_4$ | 70·7 |
| 1·0 | 71·7 |
| 1·5 | 77·5 |
| 2·0 | 71·3 |

This shows maximum action, with our amount of pepsin, in the presence of 1·5 per cent. of oxalic acid and shows, moreover, that the retarding influence of ammonium oxalate on proteolytic action, is not fully explained by the suggested neutralization of the hydrochloric acid of the gastric juice, unless it be that the ammonium chloride, formed by the reaction, effects pepsin-oxalic acid differently than it does pepsin-hydrochloric acid.

### *Sodium chloride.*

With this salt we obtained, by our method, the following results:

| NaCl. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2936 gram. | 70·64 per cent. | 100·0 |
| 0·005 per cent. | 0·2824 | 71·76 | 101·6 |
| 0·010 | 0·2896 | 71·04 | 100·5 |
| 0·025 | 0·3441 | 65·59 | 92·8 |
| 0·050 | 0·3511 | 64·89 | 91·8 |
| 0·100 | 0·3744 | 62·56 | 88·5 |
| 0 | 0·2669 | 73·31 | 100·0 |
| 0·3 | 0·4953 | 50·47 | 68·8 |
| 0·5 | 0·6175 | 38·25 | 52·1 |
| 0·8 | 0·6898 | 31·02 | 42·3 |
| 1·5 | 0·7825 | 21·75 | 29·6 |
| 3·0 | 0·8249 | 17·51 | 23·8 |

Alex. Schmidt* has recorded the retarding effect of sodium chloride on the proteolytic action of gastric juice; Petit† likewise, has stated that small quantities of sodium chloride interrupt the action of the ferment, and Wolberg‡ has recorded the same result with varying percentages of the salt; noting in addition, that very small quantities cause a slight acceleration of proteolytic action, amounting in his experiment to 2·5 per cent.

With 0·005 per cent. of the salt, we found as the results show, acceleration amounting to 1·6 per cent., larger amounts causing a gradual and proportional diminution in proteolytic action.

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* Pflüger's Archiv, xiii, p. 98.

† Jahresbericht für Thierchemie, 1880, p. 309.

‡ Pflüger's Archiv, xxii, p. 298.

Pfeiffer* has also called attention to the retarding action of this substance.

*Potassium chloride.*

With this salt our results are as follows:

| KCl. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1552 gram. | 84·48 per cent. | 100·0 |
| 0·005 per cent. | 0·1817 | 81·83 | 96·8 |
| 0·025 | 0·1550 | 84·50 | 100·0 |
| 0·050 | 0·2565 | 74·35 | 88·0 |
| 0·100 | 0·2097 | 79·03 | 93·5 |
| 0 | 0·1930 | 80·70 | 100·0 |
| 0·3 | 0·3997 | 60·02 | 74·3 |
| 1·5 | 0·6815 | 31·85 | 39·4 |
| 3·0 | 0·7282 | 27·18 | 33·6 |

The only noticeable difference between the action of this salt and the preceding, is the absence of any acceleration on the part of the potassium chloride and with larger percentages, a less vigorous retarding action.

*Ammonium chloride.*

For the sake of comparison a few experiments were tried with this salt, with the following results:

| $(NH_4)Cl$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1880 gram. | 81·20 per cent. | 100·0 |
| 0·3 per cent. | 0·4649 | 53·51 | 65·9 |
| 0·8 | 0·6615 | 33·85 | 41·7 |
| 3·0 | 0·6970 | 30·30 | 37·3 |

By looking at the table of comparisons, we see there is little constant difference in the amount of retardation caused by the ammonium, potassium and sodium salts of hydrochloric acid. This result, however, is quite different from that obtained by Wolberg, who found that ammonium chloride influenced proteolytic action but very little, while both potassium and sodium chloride caused great retardation. This difference in result, may be due to difference in strength of gastric juice or to difference in length of time the mixtures were warmed at 40° C.; certainly in our experiments, with pure anhydrous salts and 2 hours digestion, very little difference in digestive action is noticeable; throughout, sodium chloride causes a little less proteolytic action than the corresponding potassium salt, while of the ammonium salt, little is to be said except that it causes equal retardation.

* Centralbl. med. Wiss., 1885, p. 328.

Wolberg in speaking of the relative retarding action of the three sodium salts of hydrochloric, nitric and sulphuric acids, states that the sulphate retards the most, then the nitrate and lastly the chloride. We have noticed the same fact in our work and take it as additional evidence of the liberation of the acid of the salt added, the retarding influence of the salt being dependent, in part, on the digestive power of the pepsin-*acid* formed.

The following experiments,* showing the relative digestive power of the several acids in conjunction with pepsin, testify to the probability of this view.

All of the solutions contained 50 c. c. of an aqueous solution of glycerine-pepsin, each having a total volume of 100 c. c. and differing from each other only in the nature and percentage of the acid present.

In testing the digestive power of the solution, 1 gram of pure fibrin was added and the mixtures warmed at 40° C. for two hours, after which the amount digested was determined in the usual manner.

*Percentage of fibrin digested in the presence of the different percentages of acids.*

| | *0·05 %* | *0·1 %* | *0·2 %* | *0·3 %* | *0·4 %* | *0·5 %* | Control. *0·1 %* HCl |
|---|---|---|---|---|---|---|---|
| $HNO_3$†.... | 9·20 | 48·75 | 73·65 | 67·20 | 46·00 | | 87·65 |
| $H_2SO_4$..... | | 19·50 | 24·70 | 25·80 | 22·55 | 24·95 | 88·05 |
| $C_2H_4O_2$.... | | | | 3·70 | | 2·30 | 87·50 |

Comparing these results with their respective controls, figured as 100, we have the following values for sulphuric and nitric acids, expressive of their relative proteolytic power when combined with pepsin, as compared with 0·1 per cent. hydrochloric acid under similar conditions.

| Per cent. acid. | $HNO_3$. | $H_2SO_4$. |
|---|---|---|
| 0·05 | 10·5 | ---- |
| 0·1 | 55·5 | 22·1 |
| 0·2 | 84·0 | 28·0 |
| 0·3 | 76·7 | 29·1 |
| 0·4 | 52·5 | 25·6 |
| 0·5 | ---- | 28·2 |

From this it is evident, that nitric acid is most active, with our amount of pepsin, in a 0·2 per cent. solution, while sulphuric acid attains its maximum action in 0·3 per cent.; moreover, nitric acid is

* Made in this laboratory by Mr. R. W. Pinney.

† The acids are calculated as pure $HNO_3$, $H_2SO_4$, etc.

more than four-fifths as active as 0·1 per cent. hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as the hydrochloric acid; acetic acid being practically worthless. Hence it is obvious, that, the base being the same, acetates, borates and other salts, the acids of which are not capable of working with pepsin, will most readily retard gastric digestion; then of the other salts experimented with, sulphates and lastly nitrates.

A glance at the table of comparisons, shows here and there, results which manifestly accord with this view, notably lead acetate, cupric sulphate, zinc sulphate,* magnesium sulphate and potassium nitrate.

That this withdrawal of free hydrochloric from the pepsin, is only a partial explanation of the mode of action of neutral salts is plain from the fact that chlorides exert very pronounced retarding action; moreover the action of these latter as well as of the others cannot be due merely to mechanical causes, viz: the semi-saturation of the digestive fluid, since 3·0 per cent. of boracic acid stimulates instead of retarding, and even the presence of 10 per cent. of arsenic acid causes retardation amounting to only 32 per cent. According to Petit,† moreover, saccharose to the extent of 16 per cent. does not interrupt gastric digestion. Again, that the base entering into the composition of the salt has much to do, in many cases, with its retarding proteolytic action, is apparent when we compare the action of ferric chloride, manganous chloride, sodium chloride, mercuric chloride and other metallic salts. That this action is due in part to capability of combining with the proteid matter, thus rendering it non-digestible, is unquestionable.

Acceleration, produced by neutral salts has been noticed by other observers, but no explanation of the cause has been offered. It seems probable, however, that in the case of pepsin-hydrochloric acid, one plausible reason, at least, may be suggested. If, as has been mentioned, many of the neutral salts are decomposed by the acid of the gastric juice, it would follow that the addition of very small amounts of the salts would diminish slightly the percentage of free hydrochloric acid, while the acid liberated from the salt, would be entirely without deleterious action. Nearly every experimenter in this direction has employed in the preparation of gastric juice 0·2 per cent. hydrochloric acid, which is well adapted for the purpose, but the action of the acid is dependent in part on the amount of ferment. With our solution of pepsin, the following results, expressed in the percentage of fibrin

* Compare Petit, Jahresbericht für Thierchemie, 1880, p. 309.

† Jahresbericht für Thierchemie, 1880, p. 309.

digested, were obtained with different percentages of hydrochloric acid; the amount of pepsin being the same in each case.

| | | | | | |
|---|---|---|---|---|---|
| Per cent. HCl..... | *0·05* | *0·1* | *0·2* | *0·3* | *0·4* |
| Per cent. fibrin digested | 73·8 | *89·3* | 84·0 | 81·7 | 63·8 |

This shows plainly, that, with the amount of pepsin employed in our experiments, acceleration of proteolytic action on the addition of neutral salts, might in some instances be due to slight diminution of free hydrochloric acid. That this is not the sole cause, however, is plain from the fact that closely related salts do not act alike.

*Potassium bromide and Potassium iodide.*

With these two potassium salts, the following results were obtained:

| KBr. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3837 gram. | 61·63 per cent. | 100·0 |
| 0·005 per cent. | 0·3205 | 67·95 | 110·2 |
| 0·025 | 0·3035 | 69·65 | 113·0 |
| 0·10 | 0·4204 | 57·96 | 94·0 |
| 0·50 | 0·5690 | 43·10 | 70·0 |
| 1·00 | 0·6203 | 37·97 | 61·6 |
| KI. | | | |
| 0 | 0·2572 gram. | 74·28 per cent. | 100·0 |
| 0·005 per cent. | 0·2755 | 72·45 | 97·5 |
| 0·025 | 0·3421 | 65·78 | 88·6 |
| 0·10 | 0·2841 | 71·59 | 96·4 |
| 0·50 | 0·6367 | 36·33 | 48·9 |
| 1·00 | 0·7586 | 24·14 | 32·5 |

By comparison of these two series of results, we see that there is a very decided difference in the action of the two salts; potassium bromide in very small quantity, causes a decided acceleration in proteolytic action, which is wholly lacking with potassium iodide. Again, there is a very great difference in the amount of retardation produced by the two salts; thus by comparison with their respective controls we see, that while 1 per cent. of potassium bromide causes retardation in digestive action, amounting to 38·4 per cent., the same percentage of iodide causes retardation amounting to 67·5 per cent. It is to be supposed, naturally, that in the presence of large amounts of the salts, the ferment must act wholly in combination with hydrobromic and hydriodic acid respectively; that is to say, the hydrochloric acid of the gastric juice must have replaced these two acids in the potassium salts, and thus new pepsin-compounds formed, of which pepsin-hydrobromic acid is the more active digestive agent.

Putzeys,* indeed, found that he obtained practically the same proteolytic action [retarding] with pepsin and hydriodic acid, as with potassium iodide and pepsin-hydrochloric acid. The following results, showing the relative digestive action of pepsin with hydrobromic and hydriodic acid respectively, were obtained by Putzeys.

| HI. | HBr. | Digestive products formed. |
|---|---|---|
| 0·625 gram. | ---- | 15·86 per cent. |
| 0·937 | ---- | 31·33 |
| 3·310 | ---- | 2·33 |
| ---- | 0·882 gram. | 26·40 |
| ---- | 2·200 | 45·60 |
| ---- | 3·309 | 46·60 |

It is thus evident, that both hydrobromic and hydriodic acid can, to a certain extent, replace the hydrochloric acid of the gastric juice, although they are much less active than the latter acid. Moreover, hydrobromic acid is much more efficient than hydriodic in connection with the ferment, for in comparatively large doses the latter acid will completely stop proteolytic action. As a practical result, Putzeys suggests, that for therapeutic purposes, potassium bromide and iodide should be given ½ to 1 hour before meals. Our results are plainly in accord with Putzeys' observations.

The following table shows the relative influence on the proteolytic action of pepsin, of the various inorganic salts experimented with, compared with the controls, expressed as 100.

* Jahresbericht für Thierchemie, 1877, 279. De l'influence de l'iodure et du bromure de potassium sur la digestion stomacale.

*Table showing relative proteolytic action.*

| | 0·001 p. c. | 0·005 p. c. | 0·01 p. c. | 0·025 p. c. | 0·05 p. c. | 0·1 p. c. | 0·2 p. c. | 0·3 p. c. | 0·5 p. c. | 0·8 p. c. | 1·0 p. c. | 1·5 p. c. | 2·0 p. c. | 3·0 p. c. | 5·0 p. c. | 10·0 p. c. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $HgCl_2$ | 93·8 | 92·7 | ---- | ---- | ---- | 60·2 | ---- | ---- | 11·4 | ---- | 0 | ---- | ---- | ---- | ---- | ---- |
| $HgBr_2$ | ---- | 97·8 | ---- | 93·9 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $HgI_2$ | ---- | 107·4 | ---- | 95·1 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $Hg(CN)_2$ | ---- | 107·5 | ---- | 93·8 | ---- | 106·3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $CuSO_4+5H_2O$ | 104·8 | 102·8 | 97·0 | 86·1 | 85·6 | 61·2 | ---- | 31·5 | 26·4 | 23·3 | ---- | 19·8 | ---- | ---- | ---- | ---- |
| $Pb(C_2H_3O_2)_2+3H_2O$ | 104·2 | 100·5 | 101·9 | 103.0 | ---- | 97·8 | ---- | 69·6 | 32·8 | 2·8 | ---- | 0·7 | ---- | 0 | ---- | ---- |
| $SnCl_2$ | ---- | ---- | ---- | 97·5 | ---- | 69·6 | ---- | ---- | 35·9 | ---- | 24·8 | ---- | 13.3 | ---- | ---- | ---- |
| $As_2O_3$ | ---- | ---- | ---- | ---- | 103·0 | 99·3 | 102·6 | ---- | 105·1 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $H_3AsO_4$ | ---- | ---- | ---- | ---- | ---- | ---- | 101·2 | ---- | 101·6 | ---- | 102·0 | ---- | 98·2 | ---- | 90·8 | 68·1 |
| $ZnSO_4+7H_2O$ | 101·6 | 101·5 | 96·2 | 89·9 | ---- | 61·9 | ---- | 34·4 | 31·4 | 27·9 | 27·2 | 21·6 | ---- | 19·3 | ---- | ---- |
| $Fe_2Cl_6$ | 96·7 | 97·3 | 96·0 | ---- | 93·9 | ---- | ---- | 43·2 | 24·4 | 15·0 | ---- | ---- | ---- | 5·2 | ---- | ---- |
| $FeSO_4+7H_2O$ | 99·0 | 95·0 | 99·2 | 90·9 | 88·5 | 81.0 | ---- | 33·8 | ---- | 23·8 | ---- | 19·2 | ---- | ---- | ---- | ---- |
| $MnCl_2$ | 98·7 | ---- | 101·3 | 98·2 | 100·8 | ---- | ---- | 77·7 | ---- | 43·8 | ---- | 41·7 | ---- | 32·0 | ---- | ---- |
| $MgSO_4+7H_2O$ | ---- | 90·7 | 86·0 | ---- | 75·5 | 62·3 | ---- | 33·1 | 28·5 | 23·7 | ---- | 15·0 | ---- | 14·9 | ---- | ---- |
| $K_2Mn_2O_8$ | ---- | 21·3 | 0·6 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $K_2Cr_2O_7$ | ---- | ---- | 94·4 | ---- | ---- | 45·3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| KCN | ---- | 81·5 | ---- | 90·5 | ---- | ---- | ---- | ---- | 1·3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $K_4Fe(CN)_6+3H_2O$ | ---- | 93·3 | ---- | 94·5 | 64·1 | 33·0 | ---- | ---- | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $(NH_4)_2C_2O_4+2H_2O$ | ---- | ---- | 95·1 | 94.6 | ---- | 90·1 | ---- | ---- | 14·1 | ---- | 0·6 | ---- | ---- | ---- | ---- | ---- |
| $Na_2B_4O_7+10H_2O$ | ---- | ---- | ---- | ---- | 96·2 | ---- | 92·6 | ---- | 35·8 | ---- | 1·5 | ---- | ---- | ---- | ---- | ---- |
| $H_3BO_3$ | ---- | ---- | ---- | ---- | ---- | 102·1 | ---- | ---- | 102·2 | ---- | ---- | ---- | ---- | 104·2 | ---- | ---- |
| $KClO_3$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 74·3 | ---- | 40·7 | ---- | 25·0 | ---- | 17·6 | ---- | ---- |
| $KNO_3$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 64·9 | 52·6 | 39·8 | ---- | 25·9 | ---- | 15·3 | ---- | ---- |
| KCl | ---- | 96·8 | ---- | 100·0 | 88·0 | 93·5 | ---- | 74·3 | ---- | ---- | ---- | 39·4 | ---- | 33·6 | ---- | ---- |
| NaCl | ---- | 101·6 | 100·5 | 92·8 | 91·8 | 88·5 | ---- | 68·8 | 52·1 | 42·3 | ---- | 29·6 | ---- | 23·8 | ---- | ---- |
| $(NH_4)Cl$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 65·9 | ---- | 41·7 | ---- | ---- | ---- | 37·3 | ---- | ---- |
| KBr | ---- | 110·2 | ---- | 113·0 | ---- | 94·0 | ---- | ---- | 70·0 | ---- | 61·6 | ---- | ---- | ---- | ---- | ---- |
| KI | ---- | 97·5 | ---- | 88·6 | ---- | 96·4 | ---- | ---- | 48·9 | ---- | 32·5 | ---- | ---- | ---- | ---- | ---- |

### *Alkaloid salts.*

According to Petit, alkaloids do not interrupt the action of pepsin in acid solution. Wolberg, however, found that morphine chloride, strychnine, quinine sulphate and narcotine, used in very small quantities (0·5 grain to 100 c. c.), caused in every instance, excepting with quinine sulphate, retardation amounting on an average to nearly 3

per cent.; with quinine sulphate, slight acceleration was obtained. Strychnine and narcotine, in very small quantity, gave slight acceleration. Dr. H. v. Boeck* states, that quinine is without influence on the activity of pepsin.

In our experiments, using larger amounts of the alkaloid salts, retardation is very pronounced, doubtless due in part to replacement of the sulphuric acid of the salts by the acid of the gastric juice.

| Alkaloid salt. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3555 gram. | 64·45 per cent. | 100·0 |
| $(Sr)_2 . H_2SO_4 + 6H_2O$. | | | |
| 0·5 per cent. | 0·6825 | 31·75 | 49·2 |
| 1·0 | 0·8213 | 17·87 | 27·7 |
| $(Br)_2 . H_2SO_4 + H_2O$. | | | |
| 0·5 | 0·5685 | 43·15 | 66·9 |
| 1·0 | 0·7700 | 23·00 | 35·6 |
| $(At)_2 . H_2SO_4$. | | | |
| 0·5 | 0·6412 | 35·88 | 55·6 |
| $(Q)_2 . H_2SO_4 + 7H_2O$. | | | |
| 0·5 | 0·6606 | 33·94 | 52·6 |
| $(Ci)_2 + H_2SO_4 + 2H_2O$. | | | |
| 0·5 | 0·6885 | 31·15 | 48·3 |
| $(Mo)_2 . H_2SO_4 + 5H_2O$. | | | |
| 0·5 | 0·6225 | 37·75 | 58·5 |
| $(Na)_2 . H_2SO_4 + H_2O$. | | | |
| 0·5 | 0·6365 | 36·35 | 56·4 |

*Percentage retardation of the alkaloid salts* (0·5 *per cent.*)

| | |
|---|---|
| Strychnine | 50·8 |
| Brucine | 33·1 |
| Atropine | 44·4 |
| Quinine | 47·4 |
| Cinchonine | 51·7 |
| Morphine | 41·5 |
| Narcotine | 43·6 |

* Zeitschrift für Biologie, vol. vii, p. 428.

## VII.—Influence of Various Therapeutic and Toxic Substances on the Proteolytic Action of the Pancreatic Ferment. By R. H. Chittenden and Geo. W. Cummins, Ph.B.

With the proteolytic ferment of the pancreatic juice, few systematic experiments have been tried to ascertain the influence of those substances the frequency of whose use, as therapeutic or toxic agents, renders their action on this ferment a matter of no small consequence. The well known experiments of Heidenhain, Kühne and Schmidt have been confined to the action of sodium carbonate, sodium chloride and kindred salts of physiological importance. Pfeiffer* has indeed, in addition, experimented with a few of the sulphates of the alkalies and alkali-earths, but of the large number of metallic and other salts in common use, few have been tried, while the action of alkaloids and the gases occurring in the intestinal canal, has not been studied at all.

### *Method employed.*

It is a characteristic of the proteolytic ferment of the pancreatic juice, that it exercises considerable digestive power in a *neutral* solution.† In view therefore of the fact that the ferment, while in the intestinal canal, may be obliged to act in an acid-reacting medium (due to acid-proteids) as frequently as in an alkaline or neutral one, it seemed advisable in the experiments, to employ a neutral solution of trypsin. Moreover, it becomes necessary to use such a solution, if salts of the heavy metals are to be experimented with, since in alkaline solutions, carbonates or oxides of the metals would be formed and thus affect the results; still, again, the action of all substances can be best studied in neutral solution, since under such conditions, no replacements or decompositions of any kind take place to complicate the action of the substance, other than the direct action on the ferment or the proteid matter. Hence, a neutral solution has in every case been employed as being the most satisfactory, while only such substances have been experimented with, as contained no free acid or alkali, the destructive action of which is well known.

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* Centralbl. med. Wiss., 1885, p. 328.

† See Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46. Also Trans. Conn. Acad., vol. vii.

The solution of trypsin was prepared according to Kühne's* method from dried ox pancreas freed from fat; 40 grams dry pancreas in 500 c. c. 0·1 per cent. salicylic acid, neutralized and diluted to 2 litres.† In each digestion experiment, 25 c. c. of this neutral trypsin solution were used, to which was added 25 c. c. of water containing the substance to be experimented with, or in the control 25 c. c. of water alone, making the volume of the digestive mixture in each case 50 c. c. In testing the proteolytic action of the different solutions, 1 gram of pure dry, pulverized fibrin, described in the preceding article, was added and the mixtures warmed at 40° C. for six hours, after which the undigested fibrin was filtered on weighed filters, washed thoroughly and dried at 100–110° C. until of constant weight.

In this work as in the study of the salivary ptyalin and pepsin, it has been the effort mainly to ascertain the relative action of small quantities of the various salts, rather than the percentages necessary to completely stop the action of the ferment, this to our mind being much the more important.

*Mercuric chloride.*

With small percentages of this salt, the following results were obtained:

| $HgCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4495 gram. | 55·05 per cent. | 100·0 |
| 0·002 per cent. | 0·4465 | 55·35 | 100·5 |
| 0·003 | 0·4405 | 55·95 | 101·6 |
| 0·005 | 0·4562 | 54·38 | 98·7 |
| 0·025 | 0·5076 | 49·24 | 89·4 |
| 0·100 | 0·7753 | 22·47 | 40·8 |

As seen from the table, 0·1 per cent. mercuric chloride diminishes the proteolytic action of the ferment more than one-half. Its action in this percentage is more energetic than on pepsin, although the smaller quantities are proportionally far less active; in one case (0·003 per cent.) even causing acceleration.

Wassilieff‡ has studied the influence of mercurous chloride on pancreatic digestion and finds that calomel does not affect the action of the proteolytic ferment, while it does prevent the formation of putrefaction products, viz: indol, phenol, etc.

* Untersuchungen aus der physiolog. Inst. d. Universität Heidelberg, vol. i, p. 222.

† The solution was kept from putrefaction by the use of a little 20 per cent. alcoholic solution of thymol; enough of which remained dissolved in the fluid to prevent putrefaction also during the six hours digestion at 40° C.

‡ Zeitschrift für physiol. Chemie, vol. vi, p. 112.

### *Mercuric iodide and Mercuric bromide.*

These salts dissolved in sodium chloride in such proportion that the ultimate solutions contained like percentages of both salts, gave the following results:

| $HgI_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4707 gram. | 52·93 per cent. | 100·0 |
| 0·005 per cent. | 0·4780 | 52·20 | 98·6 |
| 0·025 | 0·5085 | 49·15 | 92·8 |
| 0·100 | 0·5994 | 40·06 | 75·6 |
| 0·200 | 0·6580 | 34·20 | 64·6 |
| $HgBr_2$. | | | |
| 0 | 0·4707 gram. | 52·93 per cent. | 100·0 |
| 0·005 per cent | 0·4400 | 56·00 | 105·8 |
| 0·025 | 0·4840 | 51·60 | 97·4 |
| 0·100 | 0·5721 | 42·79 | 80·8 |
| 0·200 | 0·6548 | 34·52 | 65·2 |

Aside from a slight acceleration, noticed with 0·005 per cent. of bromide, the two salts act very much alike, causing retardation of proteolytic action; less pronounced however, than that caused by mercuric chloride.

### *Mercuric cyanide.*

The action of this salt is somewhat peculiar, causing at first when in small quantity, noticeable retardation followed in the presence of larger percentages by increased proteolytic action, though still below the action of the normal solution of trypsin.

In a general way, its action on this ferment accords very nearly with its action on the amylolytic ferment of saliva and the proteolytic ferment of the gastric juice.

| $Hg(CN)_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2668 gram. | 73·32 per cent. | 100·0 |
| 0·005 per cent. | 0·3192 | 68·08 | 92·8 |
| 0·025 | 0·3209 | 67·91 | 92·6 |
| 0·050 | 0·3244 | 67·56 | 92·1 |
| 0·100 | 0·3308 | 66·92 | 91·2 |
| 0 | 0·3675 gram. | 63·25 per cent. | 100·0 |
| 0·3 per cent. | 0·4094 | 59·06 | 93·3 |
| 0·5 | 0·3880 | 61·20 | 96·7 |
| 1·5 | 0·3932 | 60·68 | 95·9 |

Its action is to be attributed mainly to the hydrocyanic acid radical, judging from the action of potassium cyanide on the one hand and the action of mercury salts on the other. There is, however, without doubt a close connection between the action of these salts and their power of combining with proteid matter in general.

### *Cupric sulphate.*

With this salt a more energetic retarding action is to be noticed, than in the case of the proteolytic ferment of gastric juice; 0·1 per cent. of the salt causing a retardation in proteolytic action of 65·7 per cent. as compared with a retardation of 38·8 per cent. in the case of pepsin.

| $CuSO_4+5H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·5035 gram. | 49·65 per cent. | 100·0 |
| 0·005 per cent. | 0·5027 | 49·73 | 100·1 |
| 0·025 | 0·5320 | 46·80 | 94·2 |
| 0·050 | 0·5856 | 41·44 | 83·4 |
| 0·100 | 0·8295 | 17·05 | 34·3 |

### *Lead acetate.*

With this salt the following results were obtained, agreeing essentially in the smaller percentages, with those obtained with cupric sulphate. In the presence of 0·1 per cent., however, retardation is far less than with the same percentage of cupric sulphate; 0·5 per cent. completely stops proteolytic action.

| $Pb(C_2H_3O_2)_2+3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4562 gram. | 54·38 per cent. | 100·0 |
| 0·005 per cent. | 0·4655 | 53·45 | 98·2 |
| 0·025 | 0·5391 | 46·09 | 84·7 |
| 0·050 | 0·5660 | 43·40 | 79·8 |
| 0·100 | 0·6410 | 35·90 | 66·0 |
| 0·500 | 1·0 | 0 | 0 |

### *Stannous chloride.*

This salt, in conformity with its action on the amylolytic ferment of saliva, causes very decided retardation in the proteolytic action of trypsin, requiring but a very small amount of the salt to completely stop the action of the ferment.

| $SnCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3875 gram. | 61·25 per cent. | 100·0 |
| 0·0005 per cent. | 0·4495 | 55·05 | 89·8 |
| 0·005 | 0·5370 | 46·30 | 75·5 |
| 0·025 | 1·0 | 0 | 0 |

### *Arsenious oxide.*

Schäfer and Böhm have experimented with arsenious acid, studying its influence on the proteolytic action of a glycerine infusion of

* Abstract in Jahresbericht für Thierchemie, 1872, p. 363.

pancreas. They find that arsenious acid is without influence on the action of the ferment. We do not know whether they added arsenious acid to a neutral or alkaline solution of the ferment, as we have seen only an abstract of their paper, but we find that the addition of very small amounts of arsenious oxide to a neutral solution of trypsin, does produce a slight retardation in the proteolytic action of the ferment. Owing to the comparative insolubility of arsenious oxide in water, only small percentages could be employed, but these show, as might be expected from the acid character of the substance, a decrease in proteolytic action.

| $As_2O_3$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4598 gram. | 54·02 per cent. | 100·0 |
| 0·001 per cent. | 0·4630 | 53·70 | 99·4 |
| 0·005 | 0·4513 | 54·87 | 101·5 |
| 0·025 | 0·4710 | 52·90 | 97·9 |
| 0·050 | 0·4723 | 52·77 | 97·6 |

### *Arsenic acid.*

This substance, in conformity with its more decided acid character, causes greater retardation than arsenious acid; which fact tends, by analogy, to make the retarding action of the latter still more probable. The results are as follows:

| $H_3AsO_4$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4598 gram. | 54·02 per cent. | 100·0 |
| 0·005 per cent. | 0·4563 | 54·37 | 100·6 |
| 0·025 | 0·4887 | 51·13 | 94·6 |
| 0·050 | 0·5264 | 47·36 | 87·4 |
| 0·100 | 0·6340 | 36·60 | 67·7 |

### *Ammonium arsenate.*

This salt in small amount, appears to increase the proteolytic action of the ferment, which fact would indicate that the retarding action of the two preceding arsenic compounds is due more to their acid character than to the arsenic contained in them.

| $(NH_4)_3AsO_4$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4598 gram. | 54·02 per cent. | 100·0 |
| 0·050 per cent. | 0·4620 | 53·80 | 99·6 |
| 0·100 | 0·4442 | 55·58 | 102·8 |
| 0 | 0·3675 | 63·25 | 100·0 |
| 0·5 | 0·4071 | 59·29 | 93·7 |

*Potassium antimony tartrate.*

This compound of antimony fails to produce with trypsin the marked acceleration, noticed with the amylolytic ferment studied.

This difference in action, since both ferments were in neutral solution, indicates a specific difference in the nature of the two ferments. Following are the results obtained:

| $K(SbO)C_4H_4O_6$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3978 gram. | 60·22 per cent. | 100·0 |
| 0·2 per cent. | 0·4105 | 58·95 | 97·8 |
| 0·5 | 0·4129 | 58·71 | 97·4 |
| 1·0 | 0·4258 | 57·42 | 95·3 |
| 1·5 | 0·5406 | 45·94 | 76·2 |

*Ferric chloride and ferrous sulphate.*

With these two salts of iron the following results were obtained:

| $Fe_2Cl_6$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·5215 gram. | 47·85 per cent. | 100·0 |
| 0·005 per cent. | 0·5431 | 45·69 | 95·4 |
| 0·025 | 0·6243 | 37·57 | 78·5 |
| 0·050 | 0·7457 | 25·43 | 53·1 |
| 0·100 | 1·0 | 0 | 0 |
| $FeSO_4+7H_2O$. | | | |
| 0 | 0·4075 gram. | 59·25 per cent. | 100·0 |
| 0·005 per cent. | 0·4548 | 54·52 | 92·0 |
| 0·05 | 0·6225 | 37·75 | 63·7 |
| 0·10 | 0·7177 | 28·23 | 47·6 |
| 0·25 | 0·7104 | 28·96 | 48·8 |
| 0·50 | 0·7219 | 27·81 | 46·9 |
| 1·00 | 0·7675 | 23·25 | 39·2 |
| 1·50 | 0·7861 | 21·39 | 36·1 |

Ferric chloride is seen to be far more energetic in its hindering action on the proteolytic ferment, than the ferrous salt. A like result was obtained with the amylolytic ferment of the saliva and to a lesser extent with pepsin-hydrochloric acid.

Bubnow* has tried experiments with both of these salts and found, as might be expected, that when present to the extent of 5 per cent. they prevented the appearance of putrefaction products (indol, phenol, etc.) but did not interfere with the action of the unorganized ferment. As no quantitative results were obtained, we can make no direct comparisons. In our experiments, it is to be remembered that putrefaction is prevented by thymol.

* Zeitschrift für physiol. Chemie., vol. vii, p. 327.

### *Manganous chloride.*

Following are the results obtained with this salt:

| $MnCl_2$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·5215 gram. | 47·85 per cent. | 100·0 |
| 0·005 per cent. | 0·5201 | 47·99 | 100·3 |
| 0·050 | 0·5139 | 48·61 | 101·6 |
| 0·100 | 0·5483 | 45·17 | 94·4 |
| 0·500 | 0·6807 | 31·93 | 66·7 |

Comparing the influence of this salt on the proteolytic action of trypsin, with the results obtained with the closely related iron salts, it is seen that the manganese salt has a far less retarding influence than the latter; a fact which agrees with what was observed in studying its action on pepsin-hydrochloric acid.

Consequently, manganese salts, in so far as they are adapted to replace iron salts as therapeutic agents, are apparently less liable to interfere with normal digestive action.

### *Zinc sulphate.*

| $ZnSO_4+7H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·5035 gram. | 49·65 per cent. | 100·0 |
| 0·005 per cent. | 0·5175 | 48·25 | 97·2 |
| 0·025 | 0·5805 | 41·95 | 84·5 |
| 0·050 | 0·6802 | 31·98 | 64·4 |
| 0·100 | 0·8301 | 16·99 | 34·2 |

The action of this salt on trypsin is, both in character and extent, similar to its action on the amylolytic ferment of saliva. Compared with pepsin-hydrochloric acid, however, the action of the salt is seen to be more energetic on the pancreatic ferment.

### *Barium chloride.*

The following results were obtained:

| $BaCl_2+2H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3710 gram. | 62·90 per cent. | 100·0 |
| 0·05 per cent. | 0·3515 | 64·85 | 103·1 |
| 0·5 | 0·3885 | 61·15 | 97·2 |
| 3·0 | 0·4986 | 50·14 | 79·7 |

With this salt a slight acceleration in proteolytic action is to be seen with 0·05 per cent., while the larger amounts cause noticeable retardation.

### *Magnesium sulphate.*

| $MgSO_4+7H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3495 gram. | 65·05 per cent. | 100·0 |
| 0·05 per cent. | 0·3541 | 64·59 | 99·3 |
| 0·5 | 0·3810 | 61·90 | 95·1 |
| 3·0 | 0·4706 | 52·94 | 81·3 |

Here retarding action is similar in extent to the action of barium chloride. Compared with zinc sulphate, the difference in action on trypsin is about the same in extent as the difference found in the action of the two salts on the amylolytic ferment of saliva. The retarding action of this salt on pancreatic digestion has been previously noticed by Pfeiffer.*

*Potassium permanganate.*

The retarding action of potassium permanganate is very pronounced. There is, however, a noticeable difference between the action of the salt on trypsin and its action on pepsin and ptyalin, the two latter being much more readily affected by the permanganate; that is, by much smaller percentages.

| $K_2Mn_2O_8$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4562 gram. | 54·38 per cent. | 100·0 |
| 0·001 per cent. | 0·4570 | 54·30 | 99·8 |
| 0·003 | 0·4608 | 53·92 | 99·1 |
| 0·010 | 0·4833 | 51·67 | 95·0 |
| 0 | 0·3675 | 63·25 | 100·0 |
| 0·1 | 0·4487 | 55·13 | 87·1 |
| 0·5 | 0·7769 | 22·31 | 35·2 |
| 1·0 | 1·0 | 0 | 0 |

*Potassium dichromate.*

| $K_2Cr_2O_7$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3978 gram. | 60·22 per cent. | 100·0 |
| 0·05 per cent. | 0·4032 | 59·68 | 99·1 |
| 0·2 | 0·4245 | 57·55 | 95·5 |
| 0·5 | 0·4693 | 53·07 | 88·1 |
| 1·0 | 0·5525 | 44·75 | 74·3 |
| 1·5 | 0·6321 | 36·79 | 61·0 |

The retarding action of this salt is proportionally greater than that of the other potassium salts, indicating that the acid has a specific action of its own. Moreover, being an acid salt, the acid radical is present in larger amount than in the neutral potassium salts experimented with.

*Potassium cyanide.*

The very pronounced acceleration in proteolytic action caused by the larger percentages of this salt is very interesting, especially when we recall the fact, that the retarding action of the same salt on the

* Centralbl. med. Wiss., 1885, p. 328.

amylolytic ferment of saliva and on the proteolytic ferment of gastric juice was equally decided, even with very small pecentages.

| KCN. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2668 gram. | 73·32 per cent. | 100·0 |
| 0·005 per cent. | 0·2790 | 72·10 | 98·3 |
| 0·025 | 0·2840 | 71·60 | 97·6 |
| 0·050 | 0·2678 | 73·22 | 99·8 |
| 0·100 | 0·2600 | 74·00 | 100·9 |
| 0 | 0·3675 | 63·25 | 100·0 |
| 0·3 | 0·2076 | 79·24 | 125·2 |
| 0·5 | 0·1985 | 80·15 | 126·7 |
| 1·0 | 0·2697* | 73·03 | 115·4 |
| 1·5 | 0·3315* | 66·85 | 105·6 |

*Potassium ferrocyanide and Potassium ferricyanide.*

With these two salts the following results were obtained:

| $K_4Fe(CN)_6+3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3045 gram. | 69·55 per cent. | 100·0 |
| 0·005 per cent. | 0·3363 | 66·37 | 95·4 |
| 0·50 | 0·3293 | 67·07 | 96·4 |
| 2·00 | 0·3753 | 62·47 | 89·8 |
| $K_6Fe_2(CN)_{12}$ | | | |
| 0·005 per cent. | 0·3268 | 67·32 | 96·8 |
| 0·05 | 0·3370 | 66·30 | 95·3 |
| 2·00 | 0·3912 | 60·88 | 87·5 |

Here, unlike the cyanide, there is no acceleration in the proteolytic action of the ferment, neither on the other hand is there very pronounced retardation; less indeed than is produced by a like percentage (2·0) of sodium chloride.

*Sodium tetraborate.*

With this salt we obtained the following results:

| $Na_2B_4O_7+10H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action |
|---|---|---|---|
| 0 | 0·4116 gram. | 58·84 per cent. | 100·0 |
| 0·05 per cent. | 0·3729 | 62·71 | 106·5 |
| 0·2 | 0·3260 | 67·40 | 114·5 |
| 0·5 | 0·2332 | 76·68 | 130·3 |
| 1·0 | 0·1860 | 81·40 | 138·3 |
| 2·0 | 0·1663 | 83·37 | 141.7 |
| 3·0 | 0·2276 | 77·24 | 131·2 |
| 5·0 | 0·3141 | 68·59 | 116·5 |

* It was impossible to wash these completely, consequently the weights are undoubtedly too high.

Here, unlike the action of the salt on the two preceding ferments studied, there is to be seen a gradual increase in proteolytic action, which is very pronounced in the presence of 2 per cent. of the salt, then gradually diminishes; although even with 5 per cent. of the crystallized salt, the action of the ferment continues to be increased far above that of the normal juice.

### *Sodium sulphate.*

Following are the results obtained:

| $Na_2SO_4+10H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3495 gram. | 65·05 per cent. | 100·0 |
| 0·05 per cent. | 0·3602 | 63·98 | 98·3 |
| 0·5 | 0·3558 | 64·42 | 99·0 |
| 2·0 | 0·3938 | 60·62 | 93·1 |
| 5·0 | 0·4360 | 56·40 | 86·7 |

The retarding action of this salt is quite pronounced, although it is noticeable that its retarding action is not equal to that of magnesium sulphate nor indeed to that of sodium chloride. Pfeiffer* has also noticed the retarding action of this salt. Weiss,† however, has stated that sodium sulphate in very small quantity increases the proteolytic activity of a pancreas extract. As we have not seen the original article we do not know whether definite percentages were employed or not; with 0·05 per cent. of the crystallized salt, under the conditions of our experiment, there was certainly no acceleration in proteolytic action.

### *Potassium chlorate and Potassium nitrate.*

| $KClO_3$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3662 gram. | 63·38 per cent. | 100·0 |
| 0·05 per cent. | 0·3991 | 60·09 | 94·8 |
| 0·50 | 0·3743 | 62·57 | 98·7 |
| 2·00 | 0·4101 | 58·99 | 93·0 |
| $KNO_3$. | | | |
| 0 | 0·3710 gram. | 62·90 per cent. | 100·0 |
| 0·05 per cent. | 0·3751 | 62·49 | 99·3 |
| 0·5 | 0·3804 | 61·96 | 98·5 |
| 2·0 | 0·4080 | 59·20 | 94·1 |
| 5·0 | 0·4473 | 55·27 | 87·8 |

* Loc. cit.

† Jahresbericht für Thierchemie, 1876, p. 177.

The effects of these two salts on the proteolytic action of the ferment are very similar, with the exception that potassium chlorate appears to be a little more energetic in action than the nitrate. Weiss* has stated that a very small quantity of potassium nitrate added to a pancreas infusion, causes slight increase in the proteolytic activity of the ferment. This, however, is not observable in our experiment with 0·05 per cent., although retardation is very slight.

### *Potassium chloride.*

This salt, unlike sodium chloride, appears to cause retardation of proteolytic action, and is without any accelerating influence whatever. The extent of its retardation, however, is not so great as in the case of sodium chloride. Following are the results obtained.

| KCl. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3662 gram. | 63·38 per cent. | 100·0 |
| 0·05 per cent. | 0·3740 | 62·60 | 98·7 |
| 0·5 | 0·3672 | 63·27 | 99·8 |
| 2·0 | 0·4331 | 56·69 | 89·4 |
| 5·0 | 0·4723 | 52·77 | 83·2 |

### *Sodium chloride.*

With this salt many previous experiments have been tried; Heidenhain,† after noting the accelerating action of sodium carbonate, tried the influence of sodium chloride and found that it increased the proteolytic power of the ferment. E. Pfeiffer, however, states that he found sodium chloride to exert a retarding influence on the proteolytic action of the pancreatic ferment. Pfeiffer‡ moreover states that he found sodium carbonate to exert its accelerating action only when present in 0·5 per cent., not in 0·24 per cent. as found by Heidenhain. Recent results§ obtained by us in another connection, however, fully substantiate Heidenhain's statements on this point. Lastly, Lindberger‖ has found that sodium chloride, which in a neutral or alkaline solution of trypsin accelerates its proteolytic action, causes in an acid solution (0·018 per cent. HCl, presumably as acid-proteid) marked retardation of ferment action, without, however,

* Loc. cit.

† Beiträge zur Kenntniss des Pancreas, Pflüger's Archiv, vol. x, p. 578.

‡ Loc. cit.

§ Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46–47. Also Trans. Conn. Acad., vol. vii.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

causing destruction of the ferment, as after dialysis the ferment was again active.

Our results accord in the main with Heidenhain's; acceleration, followed by retardation of proteolytic action as seen from the following figures.

| NaCl. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2993 gram. | 70·07 per cent. | 100·0 |
| 0·05 per cent. | 0·2635 | 73·65 | 105·1 |
| 0·2 | 0·3001 | 69·99 | 99·8 |
| 0·5 | 0·3372 | 66·28 | 94·5 |
| 1·0 | 0·3923 | 60·77 | 86·7 |
| 2·0 | 0·4352 | 56·48 | 80·6 |
| 3·0 | 0·4361 | 56·39 | 80·4 |
| 5·0 | 0·4740 | 52·60 | 75·0 |

*Potassium bromide and potassium iodide.*

The action of these two salts is wholly an accelerating one; more pronounced, however, in the case of the bromide than in the iodide. Following are the results of the experiments:

| KBr. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3881 gram. | 61·19 per cent. | 100·0 |
| 0·05 per cent. | 0·3773 | 62·20 | 101·6 |
| 0·5 | 0·3681 | 63·19 | 103·2 |
| 2·0 | 0·3795 | 62·05 | 101·4 |
| 5·0 | 0·3278 | 67·22 | 109·8 |
| KI. | | | |
| 0 | 0·3881 gram. | 61·19 per cent. | 100·0 |
| 0·05 per cent. | 0·3575 | 64·25 | 105·0 |
| 0·5 | 0·3915 | 60·85 | 99·4 |
| 3·0 | 0·3897 | 61·03 | 99·7 |

*Influence of gases on the proteolytic action of trypsin.*

Podolinski* in Heidenhain's laboratory, has shown that oxygen gas has the power of converting the zymogen of pancreas into the active ferment and that carbonic acid is without such power; moreover, that carbonic acid added to a sodium carbonate solution of pancreatin (trypsin) retards the proteolytic action of the ferment, because the normal carbonate is thus changed into acid carbonate which does not favor the action of the ferment so well as the simple salt. Hydrogen was found to be without such action. Podolinski further found that while oxygen favored the conversion of zymogen into the ferment, it did not influence the proteolytic action

* Pflüger's Archiv, vol. xiii, p. 426.

the ferment. Hydrogen was also found to be without marked action on the ferment, but carbonic acid retarded decidedly the proteolytic action of the ferment in an alkaline solution.

We have tried the influence of three gases, such as the ferment would naturally be brought in contact with in the intestinal canal, and find that they exert a marked influence on the activity of the ferment. We employed, as in the preceding experiments, a neutral solution of trypsin, and kept the solutions saturated with the gas during the experiment, by passing a constant current through the fluid contained in a small flask. As a control, air was passed through one digestive mixture, which served to keep the powdered fibrin in an equal state of agitation and thus make accurate comparison possible.

Following are the results:

| | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| Air | 0·4218 gram. | 57·82 per cent. | 100·0 |
| Hydrogen (H) | 0·3670 | 63·30 | 109·1 |
| Carbonic acid ($CO_2$) | 0·5665 | 43·35 | 74·9 |
| Hydrogen sulphide ($H_2S$) | 0·4884 | 51·16 | 88·5 |

From this it is seen that hydrogen gas causes a slight acceleration in the proteolytic action of the ferment, while carbonic acid causes marked retardation, which fact agrees with Podolinski's results, and shows moreover, that the action of the gas is the same in neutral and alkaline solutions. Hydrogen sulphide also causes retardation, although not so marked as carbonic acid.

### *Influence of alkaloid salts on the proteolytic action of trypsin.*

So far as we know, no previous experiments have been tried on this subject. Our results show, so far as our experiments extend, a greater susceptibility on the part of trypsin to the action of alkaloids than the amylolytic ferment of saliva. Moreover, with trypsin, the alkaloids in only one case cause acceleration of proteolytic action. Compared with pepsin-hydrochloric acid, however, we find that neutral solutions of trypsin are not so readily affected as the former ferment, except by one alkaloid, viz: narcotine, where retarding action is very pronounced. All of the alkaloids experimented with were in the form of pure sulphates.

### *Morphine sulphate.*

This salt exerts but little retarding action. The results are as follows:

| $(Mo)_2 . H_2SO_4+5H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3875 gram. | 61·25 per cent. | 100·0 |
| 0·05 per cent. | 0·3952 | 60·48 | 98.7 |
| 0·5 | 0·4105 | 58·95 | 96·2 |
| 2·0 | 0·4542 | 54·58 | 89·1 |

### *Atropine sulphate.*

This alkaloid is very similar to morphine in its action.

| $(At)_2 . H_2SO_4$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3875 gram. | 61·25 per cent. | 100·0 |
| 0·05 per cent. | 0·3909 | 60·91 | 99·4 |
| 0·5 | 0·4078 | 59·22 | 96·6 |
| 2·0 | 0·4619 | 53·81 | 87·8 |

Stolnikow* has stated that a very small amount of atropine sulphate is without influence on the ferment power of a glycerine extract of pancreas, but that large amounts of the alkaloid salt, considerably diminish the proteolytic action of the ferment.

### *Strychnine sulphate and Brucine sulphate.*

These two alkaloids produce more marked effects on the ferment, than the preceding. Of the two, as the results show, strychnine is more powerful in preventing proteolytic action.

| Alkaloid salt. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4511 gram. | 54·89 per cent. | 100·0 |
| $(Sr)_2 . H_2SO_4 + 6H_2O$. | | | |
| 0·05 per cent. | 0·4760 | 52·40 | 95·4 |
| 0·5 | 0·5792 | 42·08 | 76·6 |
| 2·0 | 0·6882 | 31·18 | 56·8 |
| $(Br)_2 . H_2SO_4 + H_2O$. | | | |
| 0·05 | 0·4915 | 50·85 | 92·6 |
| 0·5 | 0·5480 | 45·20 | 82·3 |
| 2·0 | 0·5452 | 45·48 | 82·8 |

### *Narcotine sulphate.*

| $(Na)_2 . H_2SO_4+H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4511 gram. | 54·89 per cent. | 100·0 |
| 0·05 per cent. | 0·5205 | 47·95 | 87·3 |
| 0·5 | 0·9943 | 0·57 | 1·0 |
| 2·0 | 1·0 | 0 | 0 |

This alkaloid causes a complete stopping of proteolytic action; nearly so, even when present to the extent of only 0·5 per cent.

* Virchow's Archiv, vol. xc, p. 435.

*Quinine sulphate, Cinchonine sulphate and Cinchonidine sulphate.*

The following table of results shows the action of these three salts:

| Alkaloid salt. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4679 gram. | 53·21 per cent. | 100·0 |
| $(Q)_2 . H_2SO_4 + 7H_2O$. | | | |
| 0·05 per cent. | 0·4811 | 51·89 | 97·5 |
| 0·5 | 0·6310 | 36·90 | 69·3 |
| 2·0 | 0·7600 | 24·00 | 45·1 |
| $(Ci)_2 . H_2SO_4 + 2H_2O$. | | | |
| 0·05 | 0·4868 | 51·32 | 96·4 |
| 0·5 | 0·6409 | 35·91 | 67·4 |
| 2·0 | 0·8327 | 16·73 | 31·4 |
| $(Cidine)_2 . H_2SO_4 + 3H_2O$. | | | |
| 0 05 | 0·4467 | 55·33 | 104·0 |
| 0·5 | 0·5977 | 40·23 | 75·6 |
| 2·0 | 0·7707 | 22·93 | 43·0 |

Cinchonidine, in the smallest percentage, causes a slight acceleration in proteolytic action; retardation is also less marked with 0·5 per cent. than in the case of the other two alkaloids. Otherwise the results are much alike.

On the opposite page is a table showing relative action of the various salts on the proteolytic power of the ferment, compared with the action of the controls expressed as 100.

O. Nasse,* by a study of the influence of various salts (inorganic) on fermentation, particularly their influence on the amylolytic action of the salivary ferment, pancreatic ferment, and the invert ferment of yeast, came to the conclusion that there is a manifest and important dependence on the part of the ferments in question, in their action, on the presence of salt molecules, and moreover, that this dependence is specific for each individual ferment; that the quantity, as well as the quality of a salt, exercises a specific influence upon each ferment.

Our own results, with still different ferments, and with a larger number of substances, both related and more varied in character, all testify to the truth of this statement, viz: that the unorganized ferments are much influenced by the presence of salts, and moreover, that there is no distinct relationship among the ferments in question in their behavior towards the various salts experimented with, as a study of the three tables of comparisons show. Thus one and the same salt may affect two ferments in quite a different manner, as seen

* Pflüger's Archiv, vol. xi, p. 157.

*Table showing relative proteolytic action.*

| | 0·0005 p. c. | 0·001 p. c. | 0·002 p. c. | 0·003 p. c. | 0·005 p. c. | 0·010 p. c. | 0·025 p. c. | 0·05 p. c. | 0·1 p. c. | 0·2 p. c. | 0·3 p. c. | 0·5 p. c. | 1·0 p. c. | 1·5 p. c. | 2·0 p. c. | 3·0 p. c. | 5·0 p. c. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $HgCl_2$ | ---- | ---- | 100·5 | 101·6 | 98·7 | ---- | 89·4 | ---- | 40·8 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $HgBr_2$ | ---- | ---- | ---- | ---- | 105·8 | ---- | 97·4 | ---- | 80·8 | 65·2 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $HgI_2$ | ---- | ---- | ---- | ---- | 98·6 | ---- | 92·8 | ---- | 75·6 | 64·6 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $Hg(CN)_2$ | ---- | ---- | ---- | ---- | 92·8 | ---- | 92·6 | 92·1 | 91·2 | ---- | 93·3 | 96·7 | ---- | 95·9 | ---- | ---- | ---- |
| $CuSO_4 + 5H_2O$ | ---- | ---- | ---- | ---- | 100·1 | ---- | 94·2 | 83·4 | 34·3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $Pb(C_2H_3O_2)_2 + 3H_2O$ | ---- | ---- | ---- | ---- | 98·2 | ---- | 84·7 | 79·8 | 66·0 | ---- | ---- | 0 | ---- | ---- | ---- | ---- | ---- |
| $SnCl_2$ | 89·8 | ---- | ---- | ---- | 75·5 | ---- | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $As_2O_3$ | ---- | 99·4 | ---- | ---- | 101·5 | ---- | 97·9 | 97·6 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $H_3AsO_4$ | ---- | ---- | ---- | ---- | 100·6 | ---- | 94·6 | 87·4 | 67·7 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $(NH_4)_3AsO_4$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 99·6 | 102·8 | ---- | ---- | 93·7 | ---- | ---- | ---- | ---- | ---- |
| $K(SbO)C_4H_4O_6$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 97·8 | ---- | 97·4 | 95·3 | 76·2 | ---- | ---- | ---- |
| $Fe_2Cl_6$ | ---- | ---- | ---- | ---- | 95·4 | ---- | 78·5 | 53·1 | 0 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $FeSO_4 + 7H_2O$ | ---- | ---- | ---- | ---- | 92·0 | ---- | ---- | 63·7 | 47·6 | ---- | ---- | 46·9 | 39·2 | 36·1 | ---- | ---- | ---- |
| $MnCl_2$ | ---- | ---- | ---- | ---- | 100·3 | ---- | ---- | 101·6 | 94·4 | ---- | ---- | 66.7 | ---- | ---- | ---- | ---- | ---- |
| $ZnSO_4 + 7H_2O$ | ---- | ---- | ---- | ---- | 97·2 | ---- | 84·5 | 64·4 | 34·2 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| $BaCl_2 + 2H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 103·1 | ---- | ---- | ---- | 97·2 | ---- | ---- | ---- | 79·7 | ---- |
| $MgSO_4 + H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 99·3 | ---- | ---- | ---- | 95·1 | ---- | ---- | ---- | 81·3 | ---- |
| $K_2Mn_2O_8$ | ---- | 99·8 | ---- | 99·1 | ---- | 95·0 | ---- | ---- | 87·1 | ---- | ---- | 35·2 | 0 | ---- | ---- | ---- | ---- |
| $K_2Cr_2O_7$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 99·1 | ---- | 95·5 | ---- | 88·1 | 74·3 | 61·0 | ---- | ---- | ---- |
| $KCN$ | ---- | ---- | ---- | ---- | 98·3 | ---- | 97·6 | 99·8 | 100·9 | ---- | 125·2 | 126·7 | 115·4 | 105·6 | ---- | ---- | ---- |
| $K_4Fe(CN)_6 + 3H_2O$ | ---- | ---- | ---- | ---- | 95·4 | ---- | ---- | ---- | ---- | ---- | ---- | 96·4 | ---- | ---- | 89·8 | ---- | ---- |
| $K_6Fe_2(CN)_{12}$ | ---- | ---- | ---- | ---- | 96·8 | ---- | ---- | 95·3 | ---- | ---- | ---- | ---- | ---- | ---- | 87·5 | ---- | ---- |
| $Na_2B_4O_7 + 10H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 106·5 | ---- | 114·5 | ---- | 130·3 | 138·3 | ---- | 141·7 | 131·2 | 116·5 |
| $Na_2SO_4 + 10H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 98·3 | ---- | ---- | ---- | 99·0 | ---- | ---- | 93·1 | ---- | 86·7 |
| $KClO_3$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 94·8 | ---- | ---- | ---- | 98·7 | ---- | ---- | 93·0 | ---- | ---- |
| $KNO_3$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 99·3 | ---- | ---- | ---- | 98·5 | ---- | ---- | 94·1 | ---- | 87·8 |
| $KCl$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 98·7 | ---- | ---- | ---- | 99·8 | ---- | ---- | 89·4 | ---- | 83·2 |
| $NaCl$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 105·1 | ---- | 99·8 | ---- | 94·5 | 86·7 | ---- | 80·6 | 80·4 | 75·0 |
| $KBr$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 101·6 | ---- | ---- | ---- | 103·2 | ---- | ---- | 101·4 | ---- | 109·8 |
| $KI$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 105·0 | ---- | ---- | ---- | 99·4 | ---- | ---- | ---- | 99·7 | ---- |
| $(Mo)_2 . H_2SO_4 + 5H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 98·7 | ---- | ---- | ---- | 96·2 | ---- | ---- | 89·1 | ---- | ---- |
| $(At)_2 . H_2SO_4$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 99·4 | ---- | ---- | ---- | 96·6 | ---- | ---- | 87·8 | ---- | ---- |
| $(Na)_2 . H_2SO_4 + H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 87·3 | ---- | ---- | ---- | 1·0 | ---- | ---- | 0 | ---- | ---- |
| $(Q)_2 . H_2SO_4 + 7H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 97·5 | ---- | ---- | ---- | 69·3 | ---- | ---- | 45·1 | ---- | ---- |
| $(Ci)_2 . H_2SO_4 + 2H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 96·4 | ---- | ---- | ---- | 67·4 | ---- | ---- | 31·4 | ---- | ---- |
| $(Cidine)_2 . H_2SO_4 + 3H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 104·0 | ---- | ---- | ---- | 75·6 | ---- | ---- | 43·0 | ---- | ---- |
| $(Sr)_2 . H_2SO_4 + 6H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 95·4 | ---- | ---- | ---- | 76·6 | ---- | ---- | 56·8 | ---- | ---- |
| $(Br)_2 . H_2SO_4 + H_2O$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 92·6 | ---- | ---- | ---- | 82·3 | ---- | ---- | 82·8 | ---- | ---- |

in the action of sodium tetraborate on the salivary ferment and on trypsin.

It is moreover, evident, from a comparison of the results obtained with the three ferments, that under the conditions of dilution, etc., with which our experiments were tried, the salivary ferment is the most sensitive to the action of the various salts, while of the other two, trypsin is as a rule most readily affected. Still, it is hardly possible to draw a direct comparison between the two proteolytic ferments, since they act under such different conditions; the fact that pepsin acts only in an acid medium and that both the strength and nature of the acid affect the activity of the ferment, introduces an additional factor which makes direct comparison in the case of the two ferments impossible. The possible reason for the slight acceleration of proteolytic action produced by several neutral salts, in the case of pepsin-hydrochloric acid, has been already referred to; but why neutral salts, which in large percentages show retarding action, should in smaller percentages added to a *neutral* solution of the ferment (ptyalin or trypsin) produce acceleration, can only be conjectured. We might assume a simple *stimulation* of ferment action by mere contact. That it may become very pronounced, is evident from the action of borax, potassium cyanide and potassium bromide in the case of trypsin and mercuric cyanide, ammonium arsenate, ferrous sulphate, potassium chlorate, sodium chloride, tartar emetic, and alkaloid salts in the case of the salivary ferment.

As to the manner in which the various salts produce retardation of ferment action, it would appear as if many of the results obtained, could be accounted for only by assuming, in addition to the views already offered, a direct influence in many cases, either destructive or hindering, on the ferment itself.

Those substances, which are particularly injurious to animal cells show in many cases no retarding action whatever, on the unformed ferments; this is particularly noticeable in the case of the arsenic compounds, which affect the ferments only as the solutions become acid. On the other hand, certain of the metallic salts are alike injurious to both and doubtless for the same reason, viz: on account of their power of combining with albuminous matter, which fact applies with equal force to the vegetable organisms (organized ferments). Nearly all germicides act injuriously on the unformed ferments. Many salts, however, well known as antiseptics, are without injurious action, except when present in large quantity; notably borax in the case of trypsin or boracic acid in the case of pepsin-hydrochloric acid.

## VIII.—Influence of Temperature on the Relative Amylolytic Action of Saliva and the Diastase of Malt. By R. H. Chittenden and W. E. Martin, Ph.B.

It has long been known that the ferment of saliva and the diastase of malt, differ from each other in the temperature best adapted to their amylolytic action. Paschutin* states that saliva ten times diluted, converts starch into dextrin and sugar most rapidly at 38° to 41° C., while the strongest action of the malt ferment occurs at 70° C.; rising slowly from 50° C. up to this temperature. According to Kühne,† the amylolytic action of salivary ptyalin is most energetic at 35° C., while the action of malt diastase is most rapid at 66° C.; ptyalin being destroyed at 60° C. Kjeldahl‡ states that the amylolytic power of diastase rapidly increases with increase of temperature up to +50° C. By 54° C., ferment action is more vigorous than at 50° C., while maximum action lies between +54° C. and +63° C. Above +63° C. amylolytic action rapidly diminishes with increase in temperature.

Exposing diastase for a long time to a temperature below 63° C. does not, according to Kjeldahl, weaken perceptibly the action of the ferment. Higher temperatures, however, cause a diminution in amylolytic power proportional to the length of time the ferment is heated; thus Kjeldahl states that long continued warming at +66° C. produces the same effect upon diastase as heating for a shorter time at +70° C. For the ptyalin of saliva, Kjeldahl finds the temperature most favorable for amylolytic action to be about +46° C. From this temperature, amylolytic action diminishes on both sides, although somewhat more rapidly by increase in temperature.

O'Sullivan§ and Brown and Heron‖ have also studied the influence of temperature on the amylolytic action of malt extract, more however with a view to ascertaining the relative proportion of products (maltose and dextrin) formed and the nature of the change involved, than any comparison of the effect of temperature on the energy of the ferment.

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* Quoted by Hoppe-Seyler, Physiologische Chemie. p. 187.

† Lehrbuch der Physiologischen Chemie, p. 20-21.

‡ Abstract in Jahresbericht für Thierchemie, 1879, p. 381-383.

§ On the action of malt-extract on starch, Journal Chem. Soc., 1876, ii, p. 125.

‖ Beiträge zur Geschichte der Stärke und der Verwandlungen derselben. Liebig's Annalen der Chemie, vol. cxcix, p. 213. Also Journal Chem. Soc.

Hüppe* likewise, has made a study of the effect of high temperatures on the two ferments, in manner similar to the experiments of Bull, Hüfner and Salkowski, not, however, to ascertain the effects of definite temperatures on ferment action, but rather to ascertain the extent to which the dry ferments can be heated without destroying their peculiar properties.

It has been our purpose to obtain by quantitative methods, definite expressions of the influence of temperature on the relative amylolytic action of the two ferments.

Our method of determining amylolytic action, is based upon the gravimetric determination of the cupric oxide-reducing power of the solution, resulting from the action of the ferment upon starch paste, according to the method of Allihn.† This gives very concise and definite results of admitted accuracy. The cupric oxide-reducing power of a solution, resulting from the amylolytic action of these two ferments, must necessarily express the degree of intensity of ferment action, since the more energetic the action, the larger the amount of sugar (maltose and dextrose) formed, with higher reducing power; while the weaker the action, the larger the amount of dextrins with lower reducing power.

The amylaceous material employed in the experiments, was purified corn starch. In each experiment 1 gram of the starch was made into a paste with 50 c. c. of boiling water, then 40 c. c. more water were added, and lastly, when everything was in readiness, 10 c. c. of the ferment solution, either saliva or malt extract; thus making a volume of 100 c. c. containing 1 per cent. of starch. In every case, the ferment was allowed to act upon the starch at the desired temperature for exactly thirty minutes, when further ferment action was at once stopped by the addition of a definite quantity of dilute acid. The ferment being thus destroyed, the solution was neutralized by adding an amount of sodium hydroxide equivalent to the acid, after which the solution was concentrated, then made up to exactly 100 c. c., and in 25 c. c., or one-fourth of the filtered fluid, the reducing bodies were determined. From the weight of metallic copper so obtained, the reducing bodies are, for the sake of comparison, calculated as dextrose, from which in turn is calculated the percentage of starch converted. Naturally the amount of starch digested, is larger than the figures indicate, since the reducing power of maltose and the dextrins is much smaller than that of dextrose, but the above method of calculation is most convenient and for comparison quite sufficient.

* Jahresbericht für Thierchemie, 1881, p. 446. Ueber das Verhalten ungeformter Fermente gegen hohe Temperaturen.

† Zeitschrift für Analytische Chemie, xxii, p. 448.

*The saliva* employed in the experiments was filtered, human mixed saliva, carefully neutralized and then diluted; 20 c. c. saliva to 80 c. c. water. As 10 c. c. of this fluid were used in each experiment, 1 gram of starch was exposed to the action of 2 c. c. of normal saliva in a dilution of 1:50.

*The malt extract* was prepared from coarsely ground, malted barley by extracting 10 grams with 200 c. c. water at 40° C. for 3–4 hours; then filtering, neutralizing and diluting to 500 c. c., a few drops of thymol being added to prevent acid fermentation. The amylolytic power of 10 c. c. of this diluted extract was a little more than that of a similar quantity of the dilute saliva. Each series of experiments, however, was made with different extracts, each of which showed considerable variation in amylolytic power.

### A. *Amylolytic action at definite temperatures.*

In all of these experiments, the 90 c. c. of fluid containing the starch paste was brought to the desired temperature by immersion in a large water-bath carefully regulated, the thermometer being immersed in the vessel containing the starch paste. In a similar manner, the ferment solution was quickly brought to the same temperature, care being taken in the latter case, that the fluid did not go beyond the requisite point. When the temperature became constant, 10 c. c. of the ferment solution were added and the action continued for thirty minutes.

The results are clearly expressed in the following series of tables:

SERIES I.*—SALIVA.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 10° C. | 0·0935 gram. | 0·1904 gram. | 17·19 per cent. |
| 20 | 0·1227 | 0·2496 | 22·46 |
| 40 | 0·1410 | 0·2872 | 25·83 |
| 50 | 0·1003 | 0·2040 | 18·35 |

SERIES II.—SALIVA.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 20° C. | 0·0891 gram. | 0·1816 gram. | 16·34 per cent. |
| 30 | 0·0945 | 0·1924 | 17·31 |
| 40 | 0·1203 | 0·2448 | 22·03 |
| 50 | 0·1419 | 0·2888 | 25·99 |
| 55 | 0·1129 | 0·2296 | 20·66 |
| 60 | 0·0451 | 0·0936 | 8·42 |
| 65 | 0·0125 | 0·0282 | 2·53 |

* It is of course understood, that the results in any one series are obtained with the same ferment solution.

SERIES III.—SALIVA.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 20° C. | 0·1177 gram. | 0·2396 gram. | 21·56 per cent. |
| 30 | 0·1273 | 0·2592 | 23·32 |
| 40 | 0·1285 | 0·2616 | 23·54 |
| 45 | 0·1174 | 0·2392 | 21·52 |
| 50 | 0·1131 | 0·2300 | 20·70 |
| 55 | 0·1029 | 0·2092 | 18·82 |
| 60 | 0·0883 | 0·1800 | 16·16* |
| 65 | 0·0354 | 0·0748 | 6·73 |
| 70 | 0·0017 | | |

SERIES IV.—SALIVA.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 25° C. | 0·0748 gram. | 0·1528 gram. | 13·75 per cent. |
| 30 | 0·0897 | 0·1828 | 16·45 |
| 40 | 0·1070 | 0·2180 | 19·62 |
| 45 | 0·0990 | 0·2016 | 18.18 |
| 50 | 0·0806 | 0·1644 | 14·79 |
| 55 | 0·0715 | 0·1460 | 13.14 |
| 60 | 0 0278 | 0·0596 | 5·36 |
| 65 | 0·0142 | 0·0328 | 2·95 |

SERIES V.—SALIVA.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch. converted. |
|---|---|---|---|
| 40° C. | 0·1062 gram. | 0·2164 gram. | 19·47 per cent. |
| 2 | 0·0611 | 0·1252 | 11·33 |

SERIES VI.—MALT EXTRACT.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 30° C. | 0·1374 gram. | 0·2800 gram. | 25.20 per cent. |
| 40 | 0·1520 | 0·3100 | 27·90 |
| 50 | 0·1606 | 0·3280 | 29·52 |
| 60 | 0·1408 | 0·2864 | 25·77 |
| 70 | 0·0544 | 0·1124 | 10·11 |
| 80 | 0·0127 | 0·0296 | 2·66 |

SERIES VII.—MALT EXTRACT.

| Temperature. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 35° C. | 0·1573 gram. | 0·3208 gram. | 28·87 per cent. |
| 45 | 0·1552 | 0·3168 | 28·51 |
| 50 | 0·1575 | 0·3212 | 28·90 |
| 55 | 0·1617 | 0·3300 | 29.70 |
| 65 | 0·0585 | 0·1200 | 10·80 |
| 75 | 0·0366 | 0·0768 | 6·91 |

* See remarks further on, in regard to this high result.

SERIES VIII.—MALT EXTRACT.

| Temperature. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 30° C. | 0·1301 gram. | 0·2648 gram. | 23·83 per cent. |
| 40 | 0·1471 | 0·2996 | 26·96 |
| 45 | 0·1542 | 0·3148 | 28·33 |
| 50 | 0·1488 | 0·3036 | 27·31 |
| 55 | 0·1320 | 0·2688 | 24·19 |
| 60 | 0·0691 | 0·1412 | 12·70 |
| 65 | 0·0654 | 0·1340 | 12·06 |

SERIES IX.—MALT EXTRACT.

| Temperature. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 30° C. | 0·1283 gram. | 0·2612 gram. | 23·50 per cent. |
| 35 | 0·1435 | 0·2924 | 26·31 |
| 40 | 0·1507 | 0·3072 | 27·64 |
| 45 | 0·1562 | 0·3188 | 28·69 |
| 48½ | 0·1573 | 0·3208 | 28·87 |
| 50 | 0·1588 | 0·3244 | 29·19 |
| 55 | 0·1445 | 0·2944 | 26·45 |
| 60 | 0·0742 | 0·1516 | 13·64 |
| 65 | 0·0561 | 0·1152 | 10·36 |

SERIES X.—MALT EXTRACT.

| Temperature. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 40° C. | 0·1419 gram. | 0·3042 gram. | 27·36 per cent. |
| 2 | 0·0299 | 0·0636 | 5·72 |

By a study of these results, it is evident that in the case of the salivary ferment, variations in amylolytic action are not very great between the temperatures of 20° and 50°, or even 55° C. With the temperatures experimented with, however, amylolytic action appears to reach its maximum, in the case of saliva, at 40°; although in one single instance, for some unaccountable reason it appeared to be greater at 50° C. With the diastase of malt on the other hand, amylolytic action reaches its maximum at 50° C., although in one instance it appeared somewhat greater at 55° C.; great variations, however, are not to be observed between the temperatures of 30° and 55° C. Brown and Heron,* working with extract of malt and pure potato starch at different temperatures, obtained results by determination of both specific rotary power and cupric oxide-reducing power, which point to the same conclusions as those obtained by us. Thus at 40° C., the malt extract having been previously heated at the same temperature for 20 minutes, these investigators found at the end of 30 minutes, as

* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also Journal Chem. Soc., 1879.

a result of the action of the malt extract on the starch $(a)j = 163{\cdot}3°$, while at 50° C. under the same conditions $(a)j = 162{\cdot}7°$ and at 60° C. $(a)j = 164{\cdot}1°$, or in a second experiment, $(a)j = 163{\cdot}7°$. These results show at 50° C. the formation of a little more maltose than at 40°, although the difference is very slight; while at 60° C. the amount of maltose formed, is less even than at 40° C. Evidently then, the maximum amylolytic action of diastase of malt takes place at temperatures far below 60° C.; even below 55° C.

At very low temperatures, there is a corresponding difference in the action of the two ferments, as is apparent from the results obtained at 2° C.; the ferment of saliva being comparatively far more active at this temperature than the ferment of malt.

Hence it is apparent throughout, that diastase requires a higher temperature than the salivary ferment, in order to act with equal vigor; at the same time it is evident that at the body temperature, say 40° C., the difference in action between the two ferments is not very great. At 80° C. the diastase of malt still acts upon starch, although only slightly; the salivary ferment, however, under the conditions of our experiments, does not act at all at 70° C. and only slightly at 65° C. With these higher temperatures, it makes considerable difference in the ultimate result, whether the ferment solution is quickly brought to the desired temperature or not, and whether it remains long at the temperature in question, before being added to the starch solution. Thus, in the action of saliva at 60° C., if the ferment be warmed quickly to *nearly* 60° C., say 59° C., and then added to the starch paste at 61° C., as was done in the case of Series III, amylolytic action is considerably greater than when the saliva is actually brought to 60° and kept there for a moment or so to be sure of its constancy. Some variation in the length of time, required to bring the ferment solution to the desired temperature, was unavoidable, and doubtless, slight variations in the results at higher temperatures, occur from this cause. It was not, however, our purpose at this time, to heat the ferment in order to induce a change in its character, but simply to prevent any alteration in the temperature of the starch mixture on addition of the ferment, so that the action of the ferment on the starch might take place at a constant temperature.

*The effects, on amylolytic action, of exposing the ferment of saliva to different temperatures for varying lengths of time.*

Brown and Heron* state that a malt extract, warmed quickly to 66° C. and then added at once to starch paste at the same temperature, differs but little, in the first stages of its action, from a malt extract heated at 60° C.; if, however, the malt extract be warmed for say 10 or 15 minutes at this temperature, previous to adding it to the starch paste, its amylolytic action is very much weakened. Evidently then, under the influence of the increased temperature, a portion of the ferment is destroyed or else changes are induced, by which the action of the ferment is modified. Results of like nature were previously obtained by O'Sullivan,† with malt extract.

With saliva, we have tried the following experiments, designed originally to throw light on the comparative destructibility of the ferment.

SERIES XI.—SALIVA.

The saliva was exposed to the designated temperature for the specified time, then added to the starch paste at the *same temperature* and its amylolytic power determined.

| Temperature. | Time of exposure. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 60° C. | 0 min. | 0·0409 gram. | 0·0852 gram. | 7·66 per cent. |
| 60 | 15 | 0·0213 | 0·0464 | 4·17 |
| 60 | 30 | 0·0210 | 0·0460 | 4·14 |

At 60° C. therefore, the coagulating point of albumin and the temperature at which ptyalin is supposed to be destroyed, it is apparent that destruction of the ferment is not complete even by 30 minutes exposure to this temperature. The peculiarity of the results, moreover, make it doubtful whether we have to do with destruction at all. If the reduced amylolytic action is due to simple destruction of the ferment, we should expect less ferment action after 30 minutes exposure than after 15 minutes; as it is, the action in the two cases is the same. A certain time, however, is required to produce the change in the character of the ferment. Similar results are shown in the following series of experiments, conducted in the same manner as the preceding, only at different temperatures.

* Loc. cit., p. 227.

† Loc. cit., p. 143.

SERIES XII.—SALIVA.

| Temperature. | Time of exposure. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 50° C. | 15 min. | 0·0773 gram. | 0·1576 gram. | 14·18 per cent. |
| 50 | 60 | 0·0765 | 0·1560 | 14·04 |
| 55 | 30 | 0·0474 | 0·0984 | 8·85 |
| 55 | 60 | 0·0414 | 0·0864 | 7·21 |

Comparing these results with those obtained at like temperatures in Series I.–III., it is seen that a few minutes exposure at the designated temperature, lowers materially the amylolytic power of the solution, while doubling the time of exposure does not materially affect the result; a fact which is not consistent with the view that diminution in amylolytic power, under these conditions, is due to gradual destruction of the ferment.

The following series of experiments, also with saliva, throw additional light on the action of high temperatures on this ferment. In these two series, the saliva was exposed to the designated temperature for the specified time, then *cooled to* 40° C. and added to the starch paste *at a like temperature.*

SERIES XIII.—SALIVA.

| Temperature. | Time of exposure. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 40° C. | 0 min. | 0·1081 gram. | 0·2200 gram. | 19·80 per cent. |
| 50 | 30 | 0·1026 | 0·2088 | 18·79 |
| 55 | 30 | 0·0986 | 0·2008 | 18·07 |
| 60 | 30 | 0·0279 | 0·0596 | 5·36 |

SERIES XIV.—SALIVA.

| Temperature. | Time of exposure. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|---|
| 40° C. | 0 min. | 0·1062 gram. | 0·2164 gram. | 19·47 per cent. |
| 55 | 180 | 0·0798 | 0·1628 | 14·65 |

It would appear from these results, that by exposure of the saliva to 50° or 55° C., in the latter case for even 3 hours, and then cooling to 40° C. and testing the amylolytic power of the ferment at that temperature, less diminution of ferment action is to be observed. This speaks still more strongly against destructive action, by simple coagulation, and at the same time suggests that not only does exposure to say 55° C. affect the character of the ferment, but also that the action of the ferment so treated, is in a given time different at that same temperature from what it is at 40° C., or the temperature of maximum action. This latter point, however, which is contrary to the law laid down by Brown and Heron* for malt extracts at tem-

* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also, Journal Chem. Soc., 1879.

peratures above 50° C. we reserve for further investigation. Owing to the great difficulty in rendering saliva perfectly neutral, it is possible that the observed low result at 55° C. in Series XII. may be due to the presence of a trace of either acid or alkaline carbonate.

Finally, it is to be observed that the majority of the results obtained, indicate that the influence of different temperatures, on the amylolytic action of the salivary ferment, is due rather to change in the character of the ferment, than to the direct influence of the various degrees of heat upon the cleavage of the starch molecule; similar in character to that indicated by the work of O'Sullivan, and also of Brown and Heron in the case of malt diastase.

## IX.—Influence of Bile, Bile Salts and Bile Acids on Amylolytic and Proteolytic Action.* By R. H. Chittenden and Geo. W. Cummins, Ph.B.

The influence of bile and bile acids on the digestive processes of the intestinal canal has long been considered an important one, still few experiments have been made to determine the exact influence of these substances by themselves on ferment action. The form in which the main constituents of the bile exist in the intestinal canal depends naturally upon the reaction of the contents of the intestines. If these have an acid reaction, bile acids must be present; if alkaline, salts of these acids; and it is fair to presume that under these two conditions the presence of bile may be productive of different effects on ferment action. Recorded observations tend to show that ordinarily the contents of the intestines possess a distinct acid reaction; thus Schmidt-Mülheim† has found that in dogs fed on albuminous matter, the contents of the small intestines are invariably acid, although the mucous membrane sometimes possesses an alkaline reaction. It is evident that in such cases the alkali of the bile must have combined with the acid of the chyme, which would be followed by liberation of the bile acids and partial precipitation of the same in combination with the proteid matters of the chyme. Moreover, the recorded observations of Schmidt-Mülheim tend to show that this acid condition of the contents of the intestines persists throughout the entire length of the intestinal canal. Uffelmann‡ has likewise found, in corroboration of the above, that the faeces of infants naturally nourished possess a weak acid reaction, while, on the other hand, Nothnagel,§ as a result of 800 observations, finds that human excrement, in the case of adults, varies decidedly in its reaction, being generally alkaline, more rarely acid or neutral. It is hardly proper, therefore, to conclude that it is only necessary to study the

* Also published in the American Chemical Journal, vol. vii, p. 36.

† Archiv für Physiologie, DuBois Reymond, 1879, p. 56.

‡ Jahresbericht für Thierchemie, 1881, p. 305.

§ Jahresbericht für Thierchemie, 1881, p. 309.

influence of the bile acids in their free condition on ferment action, since in the passage of the ferments through the intestinal canal there are times, doubtless, when the reaction of the mass is more or less alkaline, especially in the small intestines, for some distance beyond the opening of the bile and pancreatic ducts. In either case it is an interesting point to ascertain whether the bile salts have an action at all analogous in kind or extent to that of the free acids.

Many observations* are recorded concerning the duodenal precipitate formed in the duodenum by the action of bile on the acid-reacting chyme. The precipitate itself has generally been supposed to consist of a mixture of syntonin, peptone and bile acids, but recent experiments of Maly and Emich† with pure bile acids tend to show that only the non-peptonised albuminous bodies are precipitated, viz: coagulable albumin and syntonin, and these only by taurocholic acid, while peptone and "propeptone" remain in solution. This fact lends favor to the view advanced by Hammarsten, that the object of the precipitation of albuminous matter on the walls of the intestines is to prevent its too rapid passage through the intestinal canal, thus giving ample opportunity for the action of the pancreatic juice.

The addition of taurocholic acid to a solution of peptone, Maly and Emich find, is followed by the formation of a distinct opalescence or fine dust-like precipitate, slowly changing to fine droplets. This precipitate, however, which is doubtless the same as observed by Hammarsten and Brücke on the addition of bile to portions of a digestive mixture, does not contain according to Maly and Emich, any peptone, but consists of taurocholic acid, possibly in a modified form.

Both of these precipitations, however, would tend to mechanically throw down, to a greater or less extent, any ferment present, and thus diminish ferment action; but, as Maly points out, the main reason for a diminished action, in the case of pepsin, is to be sought for, not in a precipitation of the ferment, but in the formation of a compound of albumin with the bile acid, not digestible by pepsin-hydrochloric acid. But since this precipitation, as a normal reaction in the animal body, must take place in the intestinal canal, it is equally important to ascertain the extent of its digestibility in pancreatic juice, or, in other words, to ascertain the exact influence of bile and its several constituents on the proteolytic action of trypsin

* See Maly in Hermann's Handbuch der Physiologie, vol. v, p. 180.

† Monatshefte für Chemie, vol. iv, p. 89.

as well as on the action of pepsin and on amylolytic action: The only data bearing on these points are the recent experiments of Maly and Emich, who have found that 0·2 per cent. taurocholic acid hinders the digestive action of pepsin-hydrochloric acid, while 1 per cent. of glycocholic acid is without influence. The same investigators likewise state that 0·1 per cent. taurocholic or glycocholic acid stops the amylolytic action of the pancreas ferment, and that 0·2 per cent. taurocholic acid or 1 per cent. glycocholic acid will completely stop the amylolytic action of the salivary ferment.

Our experiments on this subject were commenced before the above results were published, and we have continued them, since we wished to ascertain likewise the influence of the bile salts, and also the effects of both salts and acids, as well as the bile itself, on the proteolytic ferment of the pancreas. The results of Maly and Emich, moreover, not being quantitative, do not express the relative effects of the various percentages of bile acids used, but simply the percentage of acid necessary to stop ferment action under the conditions described by them.

### 1.—*Influence on Amylolytic Action.*

As amylolytic ferment, we have employed filtered human mixed saliva made neutral and then diluted to a known volume. In studying the influence of the various percentages of bile salts and acids on the action of the ferment, we have used a digestive mixture (50 or 100 cc.) containing 1 per cent. of starch previously boiled with water, and 2 per cent. of saliva, together with the given percentages of bile salts or acids. The extent of diastatic or amylolytic action under the varying conditions was determined in each case by estimating the amount of reducing substances, maltose and dextrose, formed during 30 minutes warming at 40° C. Further diastatic action was at once stopped by boiling the digestive mixtures, after which they were diluted to a known volume, and the reducing substances determined in a given portion of the diluted fluid by Allihn's gravimetric method.* The reducing substances are in each instance calculated as dextrose, and the diastatic action is expressed in the percentage of starch converted into sugar.

We first tried the influence of crystallized ox bile, since bile itself contains a small amount of a diastatic ferment. A 1 per cent. solution of nicely crystallized ox bile was made, with which the following results were obtained:

* Zeitschrift für analytische Chemie, xxii, 448.

| Crystallized bile. | Wt. Cu in ⅛.* | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 per cent. | 0·0643 gram. | 0·2636 gram. | 23·72 per cent. |
| 0·01 | 0·0630 | 0·2584 | 23·25 |
| 0·02 | 0·0686 | 0·2804 | 25·23 |
| 0·03 | 0·0693 | 0·2836 | 25·52 |
| 0·05 | 0·0656 | 0·2688 | 24·19 |
| 0·10 | 0·0734 | 0·3000 | 27·00 |
| 0·20 | 0·0665 | 0·2724 | 24·51 |
| 0·35 | 0·0447 | 0·1860 | 16·74 |

Here it is plain that a mixture of sodium glycocholate and taurocholate, in such proportion as they are contained in crystallized ox bile, exerts no appreciable retarding influence on amylolytic action until present to the extent of 0·35 per cent. On the contrary, smaller percentages unmistakably tend to increase the diastatic action of the ferment. The solution of crystallized bile had, however, a slight acid reaction, and possibly this may have had some influence in giving the latter results. The saliva and starch were both neutral.

Experiments were next tried with sodium taurocholate alone, and also with sodium glycocholate. Following are the results:

| Sodium taurocholate. | Wt. Cu in ⅛. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 per cent. | 0·0787 gram. | 0·3212 gram. | 28·90 per cent. |
| 0·3 | 0·0030 | 0·0146 | 1·51 |
| 0·5 | 0·0023 | 0·0112 | 1·00 |
| Sodium glycocholate. | | | |
| 0·5 | 0·0783 | 0·3196 | 28·76 |

It is thus plainly evident that sodium taurocholate has a very decided action on the amylolytic ferment of saliva, while the same percentage of glycocholate is entirely without effect. The retarding action of crystallized bile is thus, without a doubt, due wholly to the taurocholate. Moreover, even smaller percentages of sodium taurocholate retard amylolytic action with almost equal energy.

The following results were obtained under like conditions as the preceding, except that the 2 per cent. of saliva employed was not neutralized.

| Sodium taurocholate. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 per cent. | 0·0590 gram. | 0·1212 gram. | 21·81 per cent. |
| 0·14 | 0·0079 | 0·0192 | 3·45 |
| Sodium glycocholate. | | | |
| 0·20 | 0·0758 | 0·1548 | 27·86 |

* One-eighth of the entire digestive mixture.

Thus even 0·14 per cent. of sodium taurocholate under these conditions almost entirely stops amylolytic action. The smaller percentage of glycocholate, however, causes the same increased amylolytic action observed with the smaller percentages of crystallized bile.

With the bile acids the following results were obtained. The glycocholic acid used was a nicely crystallized specimen prepared from ox bile, while the taurocholic acid, prepared from the same source, was amorphous:

| Per cent. bile acid. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·0694 gram. | 0·1420 gram. | 25·56 per cent. |
| 0·01 taurocholic. | 0·0753 | 0·1538 | 27·68 |
| 0·05 | 0·0783 | 0·1598 | 28·76 |
| 0·10 | 0·0060 | 0·0146 | 2·63 |
| 0·20 | 0 | | |
| 0·05 glycocholic. | 0·0523 | 0·1082 | 19·47 |
| 0·10 | 0·0095 | 0·0234 | 4·21 |
| 0·20 | 0·0056 | 0·0136 | 2·44 |
| 0·50 | trace | | |
| 1·00 | 0 | | |

It is thus seen that 0·1 per cent. taurocholic acid prevents amylolytic action almost entirely, while 0·2 per cent. does not allow the conversion of any starch into sugar. This agrees exactly with the results obtained by Maly and Emich.* These same investigators, however, found only a trace of amylolytic action in the presence of 0·05 per cent. taurocholic acid; a result which does not agree with what we have found, working, however, under somewhat different conditions.

The presence of 1·0 per cent. glycocholic acid entirely prevents the conversion of starch into sugar, while 0·5 per cent. allows only the smallest amount of diastatic activity. Maly and Emich likewise found that 1·0 per cent. of glycocholic acid stopped the diastatic action of saliva.

We have repeated the last series of experiments in part, using, however, normally alkaline saliva instead of neutralized.

| Per cent. bile acid. | Wt. Cu in ¼. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 | 0·0590 gram. | 0·1212 gram. | 21·81 per cent. |
| 0·1 glycocholic. | 0·0107 | 0·0258 | 4·64 |
| 0·2 | 0·0057 | 0·0139 | 2·50 |
| 0·1 taurocholic. | 0·0052 | 0·0126 | 2·26 |

* Loc. cit., p. 118.

These results agree exactly with the preceding, and both together plainly show that only small percentages of bile acids are required to entirely prevent the amylolytic action of saliva. Assuming that the amylolytic ferment of the pancreatic juice is similar in its nature to the ferment of saliva, it would follow from our experiments that whether the contents of the intestines are acid or alkaline, the presence, beyond a certain percentage, of taurocholic acid, either as free acid or as a taurocholate, would tend to diminish amylolytic action. Very small percentages, however, would have little, if any, retarding effect, indeed might increase amylolytic action. As to glycocholic acid, the free acid is much more powerful in its action on the amylolytic ferment than the sodium salt of the acid.

Considering these results in the light of a possible application to changes in the intestinal canal, it becomes an interesting point to ascertain whether bile itself exerts the same influence on amylolytic action as the bile salts. Moriggia and Battistini * state that while bile mixed with chyme gives a precipitate which, among other things, contains mucin, bile acids and pepsin, thus hindering gastric digestion, it does not, on being mixed with saliva, hinder its amylolytic action. This they found to be the case both with bile containing mucin and with bile from which the mucin had been removed by acidifying. We have, therefore, made the following experiments with fresh ox bile containing 7·46 per cent. of solid matter. The digestive mixtures contained as before 1 per cent. of starch, 2 per cent. of neutral saliva, and were warmed at 40° C. for 30 minutes:

| Ox bile. | Wt. Cu in ½. | Total amount reducing bodies. | Starch converted. |
|---|---|---|---|
| 0 per cent. | 0·0753 gram. | 0·3072 gram. | 27·64 per cent. |
| 2·0 | 0·0875 | 0·3568 | 32·11 |
| 5·0 | 0·0690 | 0·2824 | 25·41 |
| 10·0 | 0·0719 | 0·2944 | 26·50 |
| 20·0 | 0·0770 | 0·3144 | 28·30 |

Here in close accord with what has been found before, the presence of a small percentage of bile causes increased amylolytic action; larger percentages, however, have little, if any, effect; certainly not such an effect as would be expected from the known action of the bile salts. The bile itself possessed to a slight extent, diastatic action; 20 c. c. of the bile (20 per cent.) converting 4·53 per cent. of the starch into sugar in 30 minutes. This, however, could hardly account for the increased amylolytic action noticed above in the pres-

* Jahresbericht für Thierchemie, 1876, p. 196.

ence of 2 per cent. of bile. Wittich* and also Hofmann have noticed the occasional diastatic action of bile, Wittich even extracting the ferment from human bile by his glycerine method. Gianuzzi and Bufalini† have shown that the action varies considerably in bile from different animals and individuals, and without any apparent dependence upon the nature of the food. Ewald‡ states that the diastatic capacity of bile appears to be slight in all cases, and is not found in bile which has stood for some time. We have found, however, in bile from several animals considerable diastatic power; thus in one sample of fresh sheep's bile, 25 c. c. (25 per cent.) converted 24·33 per cent. of starch into sugar in 30 minutes at 40° C. We have likewise found great variation in diastatic power, varying, expressed in the percentage of starch converted into sugar under the conditions described, from 4 to 24 in the case of herbivorous animals. We have also noticed in bile from sheep and oxen the presence of a small amount of sugar, or at least a substance capable of reducing Fehling's solution. In one instance the amount was not inconsiderable; 25 grams of ox bile yielding, by Allihn's method, 0·040 gram metallic copper, equal to 0·0209 gram dextrose or 0·08 per cent. Naunyn, we believe, has already claimed the presence of sugar in bile.

While we know then that bile acids and bile salts by themselves retard very decidedly the amylolytic action of ptyalin, it would appear that the retarding influence of the latter may be, in part at least, counteracted by other substances naturally present in the bile.

### 2.—*Influence on the Proteolytic Action of Pepsin.*

It has long been known that bile has a retarding action on pepsin digestion, and Maly and Emich have recently shown the percentages of bile acids necessary to bring the action of pepsin to a standstill. We have, however, in addition, experimented with bile itself, and as in the case of the amylolytic ferment, have endeavored to study the influence of the bile acids quantitatively. The method employed for measuring proteolytic action is one frequently used in this laboratory, and which has invariably given satisfactory results. The only feature which calls for description is the preparation of the proteid matter to be digested. The material consists of carefully selected

* Jahresbericht für Thierchemie, 1872, p. 243.

† Jahresbericht für Thierchemie, 1876, p. 197.

‡ Lectures on digestion, Amer. ed., p. 77.

and thoroughly washed blood fibrin. All soluble matters are removed by successive extraction with boiling water, cold and boiling alcohol, and finally with cold and warm ether. The fibrin is thus obtained in a perfectly friable condition and can be easily ground to a coarse powder. It is then dried at 100–110° C. This material is well adapted for quantitative experiments with pepsin-hydrochloric acid; the residue remaining after a digestion can be rapidly filtered with the aid of a pump, and can be easily freed, by washing, from peptones and other soluble products of digestion.

The gastric juice employed in the experiments, consisted of a hydrochloric acid solution of a glycerine extract of the mucous membrane from a pig's stomach, in the proportion of 10 grams glycerine extract to 1 litre of 0·2 per cent. hydrochloric acid. 50 or 100 c. c. of this pepsin-hydrochloric acid were employed in each experiment, to which was added 1 or 2 grams of the dried fibrin (2 per cent.), together with the given percentage of bile or bile acids.

We first tried the influence of bile itself, using fresh ox bile, slightly alkaline in reaction and containing 10·02 per cent. of solid matter.

The digestive mixtures were warmed at 40° C. for two hours, then filtered at once, and the undigested residue washed thoroughly,* and dried at 100° C. until of constant weight. Following are the results of the first series of experiments, with 2 grams of fibrin and 100 c. c. of gastric juice.

| Bile in digestive mixture. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·1957 gram. | 90·21 per cent. |
| 0·25 | 0·1890 | 90·55 |
| 0·50 | 0·2050 | 89·75 |
| 1·00 | 0·2234 | 88·83 |
| 3·00 | 0·5453 | 72·73 |
| 5·00 | 0·7642 | 61·84 |

* In all of the pepsin-hydrochloric acid digestions the presence of bile or bile salts naturally causes more or less of a precipitate, dependent in amount upon the percentage of bile and also upon the amount of digestive products. In washing the undigested fibrin it was of course necessary to remove this precipitate. This was accomplished by pouring over the precipitate on the filter 50 c. c. of 0·5 per cent. potassium hydroxide and then washing with water until the alkali was wholly removed.

The following experiment shows that under these conditions the alkali affects the swollen fibrin but little, if any. Two portions of fibrin of 2 grams each were warmed with 100 c. c. of 0·2 per cent. HCl for 30 minutes, then filtered and one washed with water alone, the other with water and alkali. The first gave 1·9272 grams dried residue, the other 1·9155 grams.

A second series, tried under the same conditions, but with larger percentages of bile gave the following results:

| Bile in digestive mixture. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·1979 gram. | 90·10 per cent. |
| 0·25 | 0·2456 | 87·72 |
| 0·50 | 0·1927 | 90·36 |
| 9·00 | 1·1955 | 40·22 |
| 13·00 | 1·6611 | 16·94 |
| 16·50 | 1·7812 | 10·94 |
| 20·00 | 1·9241 | 3 29 |

From these two series of experiments it is evident that the presence of bile, from 1 per cent. upward, causes diminished proteolytic action, the retarding effect being proportionate to the amount of bile present. 20 per cent. of bile stops the action, under these conditions, almost completely. It is fair to presume, therefore, that the reflux of but a small amount of bile into the stomach would be productive of a diminished proteolytic action.

These results, therefore, agree with the older statements of Brücke, Hammarsten and others, to the effect that bile added to a gastric digestion has the effect of bringing the proteolytic action to a standstill. We next tried the influence of the individual bile acids with the following results:

| Taurocholic acid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·1311 gram. | 86·89 per cent. |
| 0·025 | 0·1461 | 85·39 |
| 0·050 | 0·2200 | 78·00 |
| 0·100 | 0·2421 | 75·79 |
| 0·200 | 0·2668 | 73·32 |
| 0·500 | 0·3579 | 64·21 |

Here it is seen that the smallest percentage of taurocholic acid added, produces a distinct effect on proteolytic action, and in the next series of experiments still smaller percentages of acid cause an equally marked effect. In both series of experiments, the mixtures were warmed at 40° C. for 1 hour and 30 minutes.

| Taurocholic acid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·1499 gram. | 85·01 per cent. |
| 0·010 | 0·1819 | 81·81 |
| 0·015 | 0·1900 | 81·00 |
| 0·020 | 0·2947 | 70·53 |
| 0·050 | 0·3110 | 68·90 |

Adding taurocholic acid to the digestive mixture in the form of a sodium salt has the effect of diminishing still further the action

of the ferment; doubtless, due in part to the percentage of free hydrochloric acid being diminished by decomposition of the taurocholate.

| Taurocholic acid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·2059 gram. | 79·41 per cent. |
| 0·1 | 0·6198 | 38·02 |
| 0·2 | 0·6426 | 35·74 |
| 0·5 | 0·6475 | 35·25 |

Maly and Emich found that 0·2 per cent. taurocholic acid entirely stopped the action of pepsin; in our experiments, however, ferment action was still manifest even in the presence of 0·5 per cent. of the acid. Whether this difference in result is due to difference in the acid used, or to difference in method, we cannot say. Glycocholic acid we found to be entirely without influence on the action of pepsin, as did also Maly and Emich.

### 3.—*The Proteolytic Action of Trypsin in Neutral, Alkaline and Acid Solutions.*

The trypsin solution was prepared according to Kühne's method,* from dried pancreas freed from fat; the solution after neutralization always contained some sodium salicylate, sufficient to prevent putrefaction during short digestive periods. According to Kühne,† trypsin acts quite energetically, both in neutral and in salicylic acid solutions, but most energetically when the pancreatic solution contains 0·3 per cent. sodium carbonate. According to Heidenhain,‡ the action of definite percentages of sodium carbonate varies with the amount of ferment.

We have tried quantitative experiments as a preliminary to studying the influence of bile, with the following results;§ the mixtures were warmed at 40° C. for 3 hours and 40 minutes, and contained 2 per cent. of fibrin.

| Reaction of the fluid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| neutral | 0·2312 gram. | 76.88 per cent. |
| 0·1 per cent. $Na_2CO_3$ | 0·1570 | 84·30 |
| 0 2 | 0·0925 | 90·75 |
| 0·3 | 0·0772 | 92·28 |
| 0·4 | 0·0426 | 95·74 |
| 0·5 | 0·1038 | 89·62 |
| 0·1 pr. ct. salicylic acid | 0·5651 | 43·49 |

* Untersuchungen aus der physiolog. Inst. d. Universität Heidelberg, vol. i, p. 222.

† Ibid, p. 223.

‡ Pflüger's Archiv, vol. x, p. 576.

§ The pancreatic juice was prepared from 20 grams dry pancreas, and finally diluted to 1000 c. c. 50 c. c. were used in each digestion with 1 gram of pure fibrin.

With a larger percentage of fibrin and a longer period of digestion the results are somewhat different. The following were obtained with 4 per cent. of fibrin in 6 hours and 40 minutes at 40° C.:

| Reaction of the fluid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| neutral | 0·3785 gram. | 62·15 per cent. |
| 0·1 per cent. $Na_2CO_3$ | 0·2581 | 74·19 |
| 0·2 | 0·1395 | 86·05 |
| 0·3 | 0·1588 | 84·12 |
| 0·4 | 0·1629 | 83·71 |
| 0·5 | 0·1318 | 86·82 |
| 0·1 pr. ct. salicylic acid | 0·4738 | 52·72 |

An average of the two series of results plainly shows that there is but little difference in digestive action in the presence of 0·2–0·5 per cent. sodium carbonate, although in a given solution a change in the percentage of alkali is at once manifest, to a slight extent, in the amount of fibrin digested. Greatly increased percentages of alkaline carbonate materially diminish the action of the ferment, as the following series of experiments indicate; the mixtures were warmed for 2 hours at 40° C.:

| Reaction of the fluid. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| neutral | 0·5863 gram. | 41·37 per cent. |
| 0·5 per cent. $Na_2CO_3$ | 0·1584 | 84·16 |
| 1·0 | 0·3760 | 62·40 |
| 2·0 | 0·7010 | 29·90 |
| 3·0 | 0·7892 | 21·08 |
| 4·0 | 0·8373 | 16·27 |
| 5·0 | 0·8608 | 13·92 |

The difference in action between a neutral trypsin solution and a solution containing salicylic acid is quite noticeable, at the same time it is evident that in the acid-reacting fluid the ferment simply acts more slowly, and if time be given, the action will approach more closely to that of the neutral solution. It is of course understood that the salicylic acid in the above experiments does not exist in a free state, but in combination with the proteid matter present, and doubtless in most of the experiments recorded, where trypsin has been exposed to the action of small fractions of a per cent. of acid, no free acid has been present, but only varying percentages of acid-proteids.* Kühne† has pointed out that hydrochloric acid above 0·05 per cent. is injurious to the action of trypsin, and Heidenhain‡ has

* See Danilewsky. Centralbl. med. Wiss., 1880.

† Verh. Naturhist. med. Vereins zu Heidelberg, 1877, p. 193.

‡ Pflüger's Archiv, vol. x, p. 578.

shown that the addition of 0·1 per cent. hydrochloric acid to an aqueous extract of the pancreas stops its action. C. A. Ewald* however, found that while pepsin-hydrochloric acid destroyed trypsin, trypsin could digest fibrin in the presence of 0·3 per cent. hydrochloric acid. Mays† likewise found that trypsin digestion could take place in the presence of 0·3 per cent. hydrochloric acid, but only when a relatively large proportion of fibrin was present, and in corroboration of Kühne's statement, he showed that trypsin could be destroyed both by pepsin and dilute hydrochloric acid. Engesser‡ found that a pancreatic juice did not lose its tryptic power by two hours warming with a gastric juice containing 0·5 per cent. hydrochloric acid. Langley,§ on the contrary, has shown that a glycerine extract of the pancreas loses a very appreciable amount of trypsin when warmed for two and a half hours with 0·05 per cent. hydrochloric acid. Lindberger,‖ working with an alcohol precipitate from a glycerine extract of ox pancreas, in which there would naturally be present but a small amount of proteid matter in addition to the trypsin, found that in the presence of 0·1 per cent. hydrochloric acid the ferment was entirely without action, and even in the presence of 0·012 per cent. hydrochloric acid, fibrin was much more slowly dissolved than by a neutral trypsin solution. Lindberger, moreover, found that weaker acids, as acetic and lactic, had a much different effect than the stronger hydrochloric acid; thus with dilute acetic acid, digestion of the fibrin was almost as rapid as with a neutral solution of trypsin, while with small amounts of lactic acid, ferment action was even more energetic than in a neutral solution. There is, however, no guarantee that in these experiments free acid was present.¶

We have found that *free* acids, even when present in small percentages, completely stop the proteolytic action of trypsin, and that when considerable albuminous matter is present, the action of trypsin is much hindered by the addition of acid to a neutral solution, even before the proteid matters present are saturated with acid. 0·1 per cent. *free* salicylic acid, in the presence of proteids already satu-

* Jahresbericht für Thierchemie, 1880, p. 297.

† Untersuchungen a. d. physiolog. Inst. d. Univ. Heidelberg, vol. iii, p. 378, 1880.

‡ Jahresbericht für Thierchemie, 1880, p. 297.

§ Journal of Physiology, vol. iii, No. 3.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

¶ We have seen only the abstract of Lindberger's paper, so cannot speak positively on this point.

rated with the acid, allows no proteolytic action whatever. Furthermore, a sufficient amount of proteid matter just saturated with hydrochloric acid, no free acid being present, will almost completely stop the action of trypsin. Proteid matter, however, only partially saturated with acid has a much smaller retarding action. This, doubtless, was the condition of the mixtures in Mays' and Engesser's experiments above referred to, for, as Mays states, the ferment could act in the presence of 0·3 per cent. hydrochloric acid only when a relatively large proportion of fibrin was present.

A pancreatic juice prepared from 20 grams of dried pancreas by warming it at 40° C. with 200 c.c. 0·1 per cent. salicylic acid, etc., was finally made exactly neutral and diluted to 500 c.c.; 25 c.c. of this solution required 7·5 c.c. of a 2·0 per cent. solution of salicylic acid to completely saturate the proteids present,* the excess of free acid necessary to give the tropæolin reaction being deducted.

Three digestive mixtures were made as follows:

1. 25 c.c. of the neutral pancreatic solution + 50 c.c. water.

2. 25 c.c. of the same pancreatic solution + 7·5 c.c. 2·0 per cent. salicylic acid solution + 17·5 c.c. water. The mixture was acid to test papers, but gave no reaction with tropæolin 00. It therefore contained no free acid, but 0·3 per cent. of combined acid.

3. The same as No. 2, but 2·5 c.c. more of 2·0 per cent. salicylic acid, so that the solution contained, in addition to the acid proteids, 0·1 per cent. free salicylic acid.

One gram of fibrin was added to each of these and the mixtures warmed at 40° C. for 8 hours and 40 minutes. No. 1 digested 88·34 per cent. of the fibrin, No. 2, 13·44 per cent., while No. 3 had no action whatever.

Much smaller percentages of combined salicylic acid cause an equally diminished proteolytic action; thus, in the case of a carefully dialyzed juice where the proteid matter was much diminished, the digestive mixture, with its proteids wholly saturated, contained but 0·060 per cent. of combined salicylic acid; yet this mixture, in 15 hours at 40° C. digested but 17·10 per cent. of fibrin, while the same amount of the neutral trypsin solution digested 57·80 per cent.

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* Tested by tropæolin 00 according to the method of Danilewsky (Centralbl. med. Wiss., 1880). One drop of a solution containing 0·028 per cent. free salicylic acid gives a reddish-violet color, which is, however, not permanent as in the case of hydrochloric acid, but transient. With hydrochloric acid, one drop of a 0·003 per cent. solution will give the reaction.

Combined hydrochloric acid has a greater hindering action than salicylic acid, as the following results show:

| Pancreatic solution of trypsin. | Fibrin digested in 18 hours. |
|---|---|
| neutral | 57·80 per cent. |
| 0·034 per cent. combined HCl + no free HCl | 3·90 |
| 0·034 " " HCl + 0·005 per cent. free HCl | 2·31 |
| 0·034 " " HCl + 0·010 " " | 0·87 |

It is thus evident that in an ordinary digestive mixture, or even where albuminous matter is present only in limited quantity, the addition of hydrochloric or salicylic acid to a neutral solution of trypsin reduces its proteolytic action to a minimum before any free acid is present.

### 4.—*Influence of Bile, Bile Salts and Bile Acids on the Proteolytic Action of Trypsin.*

The addition of bile to a *neutral* pancreatic juice causes but little change in its proteolytic action, as is seen from the following results obtained with ox bile containing 8·3 per cent. solid matter:

| Bile. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·4118 gram. | 59·82 per cent. |
| 1·0 | 0·3907 | 60·93 |
| 10·0 | 0·3938 | 60·62 |

A slightly increased action is the only effect produced on the trypsin.* The addition of bile to an *alkaline* pancreatic juice does not produce any very different results. The following were obtained with a pancreatic juice containing 0·3 per cent. sodium carbonate and fresh ox bile containing 10·02 per cent. solid matter:

| Bile. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·3056 gram. | 69·44 per cent. |
| 0·25 | 0·3074 | 69·26 |
| 0·50 | 0·3488 | 65·12 |
| 1·00 | 0·3633 | 63·67 |
| 5·00 | 0·3278 | 67·22 |
| 10·00 | 0·3603 | 63·97 |

Here there is no increased proteolytic action, neither is there any very great retarding effect produced. Pure sodium glycocholate and taurocholate produce results similar to bile, as the following table shows. The pancreatic juice contained 0·3 per cent. sodium carbonate:

* Compare Heidenhain, Pflüger's Archiv., vol. x, p. 579.

| Sodium taurocholate. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·2308 gram. | 76·92 per cent. |
| 0·05 | 0·2566 | 74·34 |
| 0·10 | 0·3048 | 69·52 |
| 1·00 | 0·2832 | 71·68 |
| sodium glycocholate. | | |
| 0·10 | 0·2576 | 74·24 |
| 0·20 | 0·3154 | 68·46 |

The presence of 3·0 per cent. crystallized ox bile caused a somewhat different result, increasing the proteolytic action slightly; thus, while the control, containing 0·3 per cent. sodium carbonate, digested 88·69 per cent. of fibrin, the same trypsin solution plus 3 per cent. of crystallized bile digested in the same time 89·73 per cent. of fibrin.

While bile or bile salts have but little influence on the proteolytic action of trypsin, the bile acids, even small percentages, have a much more marked effect. The following results, obtained by the addition of the bile acids to a neutral pancreatic juice, show the extent of the action:

| Bile acids. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 | 0·2516 gram. | 74·84 per cent. |
| Glycocholic, 0·03 per cent. | 0·1993 | 80·07 |
| Taurocholic, 0·10 | 0·3455 | 65·45 |
| 0·20 | 0·4332 | 56·68 |
| 0·50 | 0·4170 | 58·30 |

Here the retarding influence of taurocholic acid is very manifest, while, on the other hand, the small percentage of glycocholic acid appears to increase the action of the ferment.

In view of the possible acid-reacting character of the contents of the small intestines, it becomes an interesting point to ascertain the influence of bile on the action of trypsin in the presence of more or less combined acid. With a pancreatic juice in which the proteids were partially saturated with salicylic acid, 0·1 per cent. combined acid being present, the following results were obtained:

| Bile. | Weight of undigested residue. | Fibrin digested. |
|---|---|---|
| 0 per cent. | 0·4822 gram. | 51·78 per cent. |
| 1·0 | 0·4858 | 51·42 |
| 10·0 | 0·4091 | 59·09 |

This increased action in the presence of 10 per cent. of bile accords with Lindberger's results, this experimenter having found that bile in the presence of small percentages of (combined?) acetic and lactic acids tends to diminish the retarding effect produced by the acids alone.

In the presence of combined hydrochloric acid, the bile salts produced no effects whatever; the trypsin was entirely without action.

## X.—Absorption of Arsenic by the Brain. By R. H. Chittenden and Herbert E. Smith, M.D.

Some time since one of us* advanced the view that the amount of arsenic present in the brain, in cases of arsenical poisoning, is an index to the *form* in which the poison was taken, viz: whether in a readily soluble and diffusible form, such as sodium arsenite, or in a comparatively insoluble form, as arsenious oxide or aceto-arsenite of copper. The original experiments of Scolosuboff† on animals, with sodium arsenite, plainly showed the capability of nerve tissue for the absorption of arsenic; yet the recorded observations of toxicologists tend to show, as a rule, the presence of but traces of this metal in cases of arsenic poisoning, either acute or chronic. Scolosuboff's results are, however, undoubtedly correct; arsenic when taken in a very soluble and diffusible form without doubt does accumulate in the brain, but in our opinion *only when in that condition*, and thus in the more common forms of poisoning with the white oxide or other insoluble forms of arsenic, but a trace of the poison is to be found in the brain at any one time.

With a firm belief in the truth of the above statement, founded on personal experience and the recorded results of other workers in this field, it was maintained by one of us in a previous paper‡ that the presence of weighable amounts of absorbed arsenic in the brain may be taken as an indication of the administration of a soluble form of the poison. Experiments on animals tend to show the correctness of the theory and the results of toxical investigations, so far as our knowledge extends, contain nothing contrary to this view. If true, we ought never to find under any circumstances an accumulation of arsenic in the brain, after the administration of an insoluble form of the poison. Hence, the study of arsenic cases, where the form of poison is known, is of great importance in this connection.

We have had a recent opportunity of adding two more cases to

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* Chittenden, Amer. Chem. Jour., vol. v, p. 8; and Medico-Legal Journal, vol. ii, p. 237.

† Bulletin de la Société Chimique de Paris, vol. xxiv, p. 125.

‡ Chittenden, loc. cit.

the list of those which bear testimony to the truth of the above theory; two fatal cases of arsenic poisoning, one of which was caused by the white oxide, the other presumably by Paris green or aceto-arsenite of copper.

*Case A.*—L. K., a middle-aged laboring man, ate for his dinner at noon a quantity of bean soup. Almost immediately after, he was seized with vomiting and purging, cramps in the legs, and all the ordinary symptoms of acute arsenic poisoning. There were no marked cerebral symptoms. At 9 P. M. of the same day the patient died in a condition of collapse, having thus lived nine hours after eating the poisoned soup. An autopsy made the following day by Dr. M. C. White of the Yale Medical School, to whose courtesy we are indebted for a description of the case, and also for the organs for analysis, revealed the following points of interest: "The mucous membrane of the stomach was very much inflamed, especially around the cardiac orifice. The duodenum was likewise much inflamed, also the lower part of the rectum, showing here as a red mottled congestion. The remaining portions of the intestines were normal. The brain showed marked congestion. The kidneys were normal in appearance, the urinary bladder was nearly empty, and the mucous lining somewhat reddened. The lungs were normal, except the lower half of the right one, which was a little congested. The heart normal; small fibrinous clot in the right ventricle." In order to draw deductions of any value from the distribution of arsenic in the body of the deceased we must know positively as to the form in which the poison was taken. Fortunately, we were able to obtain the residue of the soup eaten by the deceased. Microscopic examination of the sediment plainly showed octahedral crystals of arsenious oxide, and we were able to separate from a small portion of the solid residue 24 milligrams of the oxide. Plainly the soup was poisoned by simply mixing with it arsenious oxide in substance. As to the quantity of arsenic present in the soup we have the following data: 125 c. c., oxidized with hydrochloric acid and potassium chlorate, yielded 314·6 milligrams of arsenious sulphide, equal to 253·2 milligrams of arsenious oxide. As to the amount taken by the deceased we have no knowledge; we infer, however, since the soup constituted the main portion of his dinner, that a large quantity was eaten, which view we think is substantiated by the intensity of the vomiting and purging so characteristic of large doses of the poison.

Here then, we have an unquestionable case of poisoning with arsenious oxide, and under conditions most suitable for rapid absorp-

tion; a probable empty condition of the stomach, together with a large amount of the poison, a considerable portion of which must probably have been dissolved in the soup. Added to this, nine hours intervened between the taking of the poison and death. Certainly then everything favored an absorption of poison by the brain, if such is characteristic of this form of arsenic. Naturally the vomiting and purging would remove much of the poison, still the relative proportion of absorbed arsenic would not be materially altered, and thus if Scolosuboff's results with soluble arsenites are applicable to arsenious oxide, we ought to find in this case, in conformity with his results, a larger percentage of arsenic in the brain than in the liver or kidneys.

Following are the results actually found:*

Liver (1259 grams) contained 76·0 milligrams $As_2O_3$.
Kidney and bladder (332 grams) contained 0·6 milligram $As_2O_3$.
Brain ($\frac{1}{4}$=328 grams dry) contained simply a recognizable trace.

*Case B.*—J. G., a young woman, age unknown. Regarding the details of this case we have less definite knowledge. She was last seen alive on Friday night, at which time she threatened to poison herself. The following Monday morning she was found dead, and near her an open package of *Paris Green.* She had evidently been dead some time, and both the condition of her room and person gave evidence of excessive purging and vomiting. An autopsy by Dr. White showed an entire absence of inflammation of the alimentary tract, and also a lack of any abnormal condition sufficient to account for death. The verdict was therefore, death by poisoning with Paris green or aceto-arsenite of copper.

Through the kindness of Dr. White we were able to obtain portions of the body for analysis. The contents of the stomach were entirely free from arsenic, the poison having been wholly removed by the purging and vomiting; the trace found therefore, was the amount absorbed by the muscle tissue of the stomach. Following are the amounts of arsenic found in the parts analyzed:

Liver (2984 grams) contained 12·78 milligrams $As_2O_3$.
Kidneys and bladder (515 grams) contained 3·40 milligrams $As_2O_3$.
Muscle of thigh (735 grams) contained 0·97 milligram $As_2O_3$.
Stomach (425 grams) contained a trace.
Brain (1179 grams) contained a trace.

* The method of analysis consisted in the oxidation of the tissue with nitric and sulphuric acids, the arsenic being weighed as metallic arsenic. See Amer. Chem. Journal, vol. ii, p. 235.

The trace of arsenic in both the muscle tissue of the stomach and in the brain was very small; the entire brain could not have contained more than 0·2 of a milligram of arsenic.

These results plainly substantiate the views set forth above and lend favor to the belief that Scolosuboff's results with sodium arsenite are applicable only to that form of poison, and not to the more insoluble compounds of arsenic. These two cases, therefore, are additional evidence that in poisoning with arsenic the presence of an appreciable amount of poison in the brain, is an indication amounting almost to proof positive of the administration of a soluble and diffusible form of arsenic.

## XI.—Influence of Potassium and Ammonium Bromides on Metabolism. By R. H. Chittenden and W. L. Culbert, Ph.B.

As a question in the physiology of nutrition, it is very desirable to be able to state something definite regarding the influence of bromides upon the metabolism of the body; particularly their influence upon the metabolism of proteid matter, as shown in the excretion of urea and uric acid, and in view of their special application as therapeutic agents in diseases of the nervous system, their influence also on the decomposition of nerve substance, as shown in the excretion of phosphoric acid. Two complete investigations appear to have been made upon this subject; one in 1868 by Dr. J. H. Bill,* and one in 1883, by Dr. B. Schulze,† the results of which are more or less in direct opposition to each other. We have also seen a reference to two other investigations, quoted by Dr. Wood,‡ in which Dr. Rabuteau found the daily excretion of urea slightly lessened under the influence of bromide, as did also Dr. Bartholow.

Dr. Bill's investigation, which was a very thorough one, had for one of its objects to ascertain whether bromides reduce the amount of phosphoric acid excreted, like such known hypnotics as morphine; at the same time careful examination was made of the variations in urea and uric acid under the different conditions of the experiments. The experiments were all conducted on one person with a body weight of 160 pounds, which remained fairly constant throughout. The experiments were made in series under known conditions with uniformity of habits, diet, etc. Unfortunately, however, no data are given regarding the nature of the diet, the closeness with which it was adhered to, or whether the body was kept in a state of nitrogenous equilibrium. Each series of experiments, moreover, covers at the most, but six days; three days without bromide and three days with, consequently slight variations might easily be absorbed in an average of three results. The urea in Dr. Bill's work was deter-

* Amer. Jour. med. Sciences, July, 1868. Experimental Researches into the action and Therapeutic value of Bromide of Potassium.

† Zeitschrift für Biologie, vol. xix, p. 301. Einfluss des Bromkalium auf den Stoffwechsel.

‡ Therapeutics, p. 337.

mined with a "mercury solution," phosphoric acid with uranium solution and uric acid by precipitation and weighing as such. The results obtained were as follows: With moderate doses of potassium bromide (3·0–8·0 grams per day) urea was not affected, phosphoric acid was slightly increased, uric acid likewise, though much more decidedly; with larger doses of bromide (10·0–14·0 grams per day, continued for 2–3 days) phosphoric acid was diminished in amount, but this Dr. Bill intimates could not be attributed to regular hypnotic action, since the other urinary constituents were likewise diminished, notably the urea. Both large and small doses of bromide increased the quantity of urine passed in the twenty-four hours. This Dr. Bill asserts was not due to the increased drinking of water, for no thirst, not even with the largest doses, was ever present.

Dr. Schulze, experimenting on his own person, obtained results quite different from these. This investigator lived on a fixed diet of the following composition:

| | |
|---|---|
| 220 grams fresh meat | =7·13 grams N. |
| 50 grams air-dried wheat bread | =0·92 grams N. |
| 30 grams cocoa powder | =1·14 grams N. |
| 30 grams butter. | |
| 30 grams sugar. | |
| 5 grams salt. | |
| 1500 grams water. | |
| | 9·19 grams N. |

The average amount of nitrogen excreted daily was about 11 grams.

Taking this amount of food daily, with uniform habits of sleep, exercise, etc., Dr. Schulze states that he soon reached a point where the daily excretion of nitrogen, sulphur and phosphorus remained fairly constant. Potassium bromide was then taken three days, in divided doses of 10 grams each day. Diuretic action was very noticeable. Phosphorus was diminished, sulphur very much increased, and nitrogen (on two days) apparently slightly increased under the influence of the bromide. As, however, the increased excretion of sulphur was not accompanied with a corresponding increase in the excretion of nitrogen, Schultze considers that the increase in sulphur cannot be due to increased metabolism of simple albuminous matter, and seeks to show that the potassium bromide must have decomposed, to a slight extent, some nitrogenous phosphorized principle or principles, such as lecithin (glycerine-phosphoric acid) and nuclein, so abundant in the brain and nerve substance in general. Schulze therefore concludes that under the influ-

ence of potassium bromide there is probably a decided diminution of metabolic activity in the nervous system, accompanied by decreased nervous irritability.

By determining the nitrogen of the fæces, Schulze concluded that the bromide exercised no particular influence on the digestibility of the food.

In our experiments great care was taken first, to insure body equilibrium and then to obtain sufficient data by analysis, to be sure of the requisite constancy in the composition of the daily excretions. The experiments were tried throughout on the person of one of us (W. L. C.), of good physique and vigorous constitution. The diet was weighed out each day with scrupulous care and was as follows:

| | |
|---|---|
| Fresh meat [beef] | 142·0 grams. |
| Potatoes | 283·5 " |
| Wheat bread | 256·0 " |
| Oat meal | 50·0 " |
| Butter | 56·7 " |
| Sugar | 28·3 " |
| Salt | 0·7 " |
| Milk | 700·0 " |
| Water | 345·5 " |

This diet was commenced on the third day of April and continued for nine days before any attempt was made to ascertain the daily amounts of urea, etc., excreted. Then the urine was analyzed for nine successive days, after which doses of potassium bromide were taken. The above daily amount of food was divided into three portions and taken at the same time each day; at 7:30 a. m., 1 p. m., and 6 p. m. Exercise was taken regularly and in stated amounts; consisting of a walk each morning before breakfast and exercise with dumbbells just before retiring at 11 p. m. Care was taken not to exercise so freely as to induce perspiration. Throughout the day routine duties allowed of regular habits. It was thus found possible to keep even the minor conditions of the experiment constant throughout. The urine was collected from 7:30 a. m. of one day to 7:30 a. m. of the next, and was at once analyzed. Urea was determined by Pflüger's* modification of Liebig's method, with a standard solution of mercuric nitrate. All the precautions so carefully worked out by Pflüger; preparation of a mercuric nitrate solution of the proper specific gravity, a standard solution of sodium carbonate to neutralize the acid of the former, preliminary determination of

* Pflüger's Archiv, vol. xxi, p. 248.

chlorine and removal of the same, before precipitating the urea; were carried out with very satisfactory results. Uric acid was determined according to the older method of Heintz* with the modification of Zablins, and being conducted each time under exactly the same conditions and with a urine of approximately the same composition, the results are to be considered as strictly comparable. Chlorine and bromine were determined in the usual manner with a standard solution of silver nitrate; the results, however, are not given as they are of value only as essential to the urea determinations. Total phosphoric acid was determined by means of a standard solution of uranium nitrate.† Phosphoric acid in combination with alkali-earths was determined by precipitation with ammonium hydroxide, allowing the mixture to stand 24 hours, filtering the precipitated phosphates, washing thoroughly with diluted ammonia, then dissolving in a definite amount of dilute acetic acid and titrating with uranium solution. Total amount of solid matter contained in the 24 hours' urine, was calculated by the use of Christison's formula.

The diet specified, was commenced on the 3d day of April; on the 12th the urine was collected for analysis, the body weight taken and the investigation then carried forward without interruption. Table No. I. gives the results of the analysis of the urine for nine consecutive days, and shows the average amount of variation to be expected under the conditions of the experiment.

On the 21st of April, 60 grains of potassium bromide were taken in divided doses as seen in Table No. II. The bromide was taken about midway between the hours of eating, so that it might not affect digestion. On the 22d the dose was increased to 100 grains and then to 150, the latter amount being taken daily for three consecutive days. Table No. II. shows the effects of the bromide on the system, for the six days it was taken.

On the first day, the only apparent influence of the bromide is to cause a diminution in the amount of phosphoric acid excreted; seen both in the total $P_2O_5$ and in the $P_2O_5$ in combination with alkali-earths. On the second day, the diuretic action of the salt is apparent, accompanied with an increase in specific gravity, and a decided increase in the amount of urea excreted, together with a slight increase in the amount of uric acid. Phosphoric acid was still diminished in amount. On the third day, the body weight commenced to diminish and continued to do so throughout the experiment; diuretic

* Die Lehre vom Harn, Salkowski und Leube, p. 94–95.

† Die Lehre vom Harn, p. 184.

action was still apparent as was also increased elimination of urea, and to a slight extent of uric acid likewise. Indeed, the most noticeable effect of the bromide, next to its diuretic action is its decided influence on proteid metabolism as shown by the increased elimination of urea. As to phosphoric acid the results are not so striking, although an average of the two series shows a diminished excretion, both of total $P_2O_5$ and of $P_2O_5$ in combination with alkali-earths. The average difference in the two series of results is clearly shown by the following table:

| | Average of Table No. I, without KBr. | Average of Table No. II, with KBr. |
|---|---|---|
| Total quantity of urine | 926 c. c. | 1010 c. c. |
| Sp. Gr. | 1025, 8 | 1026, 3 |
| Total solid matters | 56·7329 grams. | 63·6252 grams. |
| Total $P_2O_5$ | 2·7540 | 2·5426 |
| $P_2O_5$ in combination with Ca and Mg. | 0·6022 | 0·5452 |
| Uric acid | 0·6752 | 0·6858 |
| Urea | 34·8681 | 35·9454 |

Our results therefore plainly indicate, that under the influence of potassium bromide, nitrogenous metabolism is increased while the excretion of phosphoric acid is slightly diminished in amount; not however, to any such extent as would be expected by an active hypnotic agent. The bromide taken, the largest doses about 10 grams per day, produced its usual physiological effects; such as drowsiness, diminution of the circulation with accompanying coldness and paleness of the skin. Constipation was not noticed while taking the bromide, but later on it became somewhat troublesome, once or twice alternating with a slight diarrhœa. In accord with Dr. Bill, we noticed an increase in the acidity of the urine while taking the bromide, as also a deepening of the color.

With bromide of potassium therefore, our results agree with those of Dr. Schulze in showing an increased excretion of nitrogen (urea and uric acid), although far more pronounced than he found in his experiments, while the diminution in phosphorus is less pronounced than found by Schulze.

With Dr. Bill's experiments our results agree in so far as the diminution of phosphoric acid is concerned, but are entirely different as regards the urea. Since, however, Dr. Bill retained uniformity in diet only during the days of the experiments, it is quite possible that lack of nitrogenous equilibrium may have had some influence on his results. The increased elimination of urea noticed in our experiments is certainly indicative of increased metabolic activity; it is,

however, suggestive that potassium bromide has been recently found to exercise a very decided accelerating influence on the proteolytic action of both pepsin-hydrochloric acid* and trypsin;† while, therefore, this fact may have something to do with the increased elimination of nitrogen, particularly as the diet used is quite rich in proteid matter, it seems more probable to suppose that the above changes in the excretion of nitrogen are due rather to changes in the tissue proteids; still it would have been interesting if the nitrogen in the fæces had been determined each day.

Although the last dose of potassium bromide was taken on the 26th of April, the same diet was still continued and the urine carefully examined daily, until the 8th of May, at which time the amount of bromine in the urine was reduced to a minimum. Dr. Bill states that bromine usually disappears entirely from the urine in ten days after the last dose of bromide.

The results of the twelve days analyses are shown in Table No. III. In examining this table it is interesting to note how quickly the elimination of urea is changed on stopping the doses of bromide. On the 26th, the last day the bromide was taken, the excretion of urea amounted to 37·5 grams; on the 27th it fell to 31·8 grams, far below what it had been any time before the bromide was taken. It would thus appear that after withdrawal of the bromide, nutrition which had been accelerated, rebounded in proportion to the preceding acceleration. Uric acid, moreover, which had likewise been increased in amount by the bromide, was now also correspondingly diminished. Furthermore, the diuretic action of the bromide was at once stopped, and the specific gravity fell to 125·5. In the case of phosphoric acid, however, the action of the bromide appears to be continued for a day or two after its withdrawal, and indeed it is noticeable throughout, that the diminution in phosphoric acid excreted, is not at all proportional to the amount of bromide taken. In fact phosphoric acid, both total $P_2O_5$ and alkali-earth $P_2O_5$, appears to be more decidedly diminished on those days when the amount of bromide in the blood was the smallest, notably on the 21st, 24th and 27th of April. By the 3d day after withdrawal of the bromide, the excretion of urea had gone nearly back to the daily amount, prior to taking the bromide; still it is to be seen in Table No. III, that the average excretion of urea, uric acid and phosphoric acid is below the average excretion recorded in Table No. I. In fact after the continued doses

* Chittenden and Allen. Trans. Conn. Acad., vol. vii.

† Chittenden and Cummins. Ibid.

of potassium bromide, the metabolism of the body did not fall back to its original height; but being temporarily accelerated during the exhibition of potassium bromide, it [the nitrogenous metabolism] fell back on withdrawal of the same, to a far lower level, and although later somewhat increased in amount, its average was still lower than recorded in Table No. I. Nutrition had evidently been disturbed; the body weight showed gradual diminution, and on the 4th and 6th of May there was slight diarrhœa accompanied with a decided decrease in the amount of urea and uric acid excreted.

Dr. Bill appears to have experimented somewhat with sodium bromide, although we find no results recorded, aside from the fact that this salt, like potassium bromide, caused an increased excretion of uric acid, and the general statement that "when taken by the mouth, bromide of sodium does not produce the same effects as bromide of potassium." In view of the increased excretion of urea, noticed under the influence of the potassium salt, we were interested in seeing whether ammonium bromide would have a like influence, especially in view of the fact that v. Schrœder* has shown that ammonium carbonate is directly convertible into urea by passage through the liver.

The physiological action of ammonium bromide is stated to resemble in many points that of potassium bromide, while in other points it differs essentially.† As to its influence on metabolism no experiments whatever appear to have been made.

On the 9th of May, 75 grains of ammonium bromide were taken, in divided doses, as shown in Table No. IV. In all, 425 grains of the salt were taken in four consecutive days. The action of the salt on the system was not as pleasant as that of potassium bromide; causing a general weakness and indisposition, a slight diminution in the pulse, an occasional cold perspiration, more marked lividity of the countenance and a parched, dry taste in the mouth. An habitual eruption of the skin was moreover much increased and accompanied with acne on the back and shoulders. Undoubtedly these disagreeable symptoms were much augmented by the temporary lassitude which was beginning to be apparent; doubtless due to the approach of warm weather together with lack of the accustomed vigorous exercise and the long continued use of the somewhat monotonous diet.

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* Archiv f. exp. Pathol., vol. xv, p. 364. Also Report on Progress in Physiological Chemistry in Amer. Chem. Jour., vol. v, p. 219.

† Wood, Therapeutics, p. 341.

TABLE I.—WITHOUT BROMIDE.

| April. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | gram. | gram. | grams. |
| 12 | 72100 | 950 c. c. | Acid. | 1026 | 59·0473 | 2·7678 | 0·6576 | 0·7443 | 35·7596 |
| 13 | 72100 | 930 | Acid. | 1026 | 57·2590 | 2·8596 | 0·6132 | 0·6901 | 35·0067 |
| 14 | 72000 | 1012 | Acid. | 1025 | 60·4227 | 2·7249 | 0·6060 | 0·6654 | 34·3699 |
| 15 | 72000 | 920 | Acid. | 1025 | 54·9298 | 2·7038 | 0·5587 | 0·6969 | 35·3247 |
| 16 | 72800 | 928 | Acid. | 1025 | 55·4074 | 2·7352 | 0·6188 | 0·7146 | 35·4567 |
| 17 | 72600 | 915 | Acid. | 1025·5 | 55·7510 | 2·6813 | 0·5946 | 0·7407 | 32·5379 |
| 18 | 72400 | 880 | Acid. | 1026·5 | 55·7755 | 2·6909 | 0·5569 | 0·5936 | 34·5360 |
| 19 | 72100 | 972 | Acid. | 1026 | 60·4147 | 2·9227 | 0·6399 | 0·6551 | 36·4960 |
| 20 | 72100 | 830 | Acid. | 1026 | 51·5887 | 2·7002 | 0·5746 | 0·5769 | 34·3255 |

TABLE II.—WITH POTASSIUM BROMIDE.

| April. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. | Amount of KBr taken, in grains. |
|---|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | gram. | gram. | grams. | |
| 21 | 72200 | 870 c.c. | Acid. | 1026 | 54·0749 | 2·2169 | 0·5542 | 0·6895 | 34·7129 | 20 at 10 a. m.<br>20 3 p. m.<br>20 10 p. m. |
| 22 | 72200 | 1005 | Acid. | 1027 | 64·9316 | 2·5268 | 0·5805 | 0·7075 | 36·0992 | 33 at 10 a. m.<br>33 3 p. m.<br>34 10 p. m. |
| 23 | 71700 | 1018 | Acid. | 1027 | 65·7715 | 2·6630 | 0·5411 | 0·6388 | 36·1708 | 50 at 10 a. m.<br>50 3 p. m.<br>50 10 p. m. |
| 24 | 71600 | 920 | Acid. | 1025·5 | 56·0557 | 2·2876 | 0·4805 | 0·6118 | 35·1002 | 50 at 10 a. m.<br>50 3 p. m. |
| 25 | 71500 | 1125 | Acid. | 1026·5 | 71·3039 | 2·9013 | 0·5096 | 0·6750 | 36·4778 | 50 at 10 a. m.<br>50 3 p. m.<br>50 10½ p. m. |
| 26 | 71000 | 1120 | Acid. | 1026 | 69·6137 | 2·6605 | 0·6056 | 0·7924 | 37·5117 | 50 at 10 a. m.<br>50 3 p. m.<br>50 10 p. m. |

TABLE III.—WITHOUT BROMIDE.

| April. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | gram. | gram. | grams. |
| 27 | 70800 | 830 c. c. | Acid. | 1025·5 | 50·5790 | 2·5779 | 0·4527 | 0·6420 | 31·8277 |
| 28 | 70400 | 840 | Acid. | 1025·5 | 51·1813 | 2·5623 | 0·5008 | 0·6846 | 32·3742 |
| 29 | 70500 | 905 | Acid. | 1025·5 | 55·1417 | 2·7606 | 0·4810 | 0·7647 | 33·3858 |
| 30 | 70400 | 945 | Acid. | 1025·5 | 59·2743 | 2·5806 | 0·4996 | 0·6693 | 33·4907 |
| May. 1 | 70400 | 985 | Acid. | 1024·5 | 57·6063 | 2·5676 | 0·5826 | 0·6363 | 34·3289 |
| 2 | 70500 | 1050 | Acid. | 1023·5 | 58·8438 | 2·5138 | 0·5435 | 0·6405 | 33·4344 |
| 3 | 70100 | 1020 | Acid. | 1024·5 | 59·6533 | 2·6682 | 0·5987 | 0·6324 | 33·8654 |
| 4 | 69800 | 835 | Acid. | 1025 | 49·8547 | 2·3155 | 0·4438 | 0·5716 | 30·6414 |
| 5 | 70100 | 905 | Acid. | 1024 | 51·8222 | 2·5849 | 0·4476 | 0·7353 | 33·1222 |
| 6 | 69600 | 905 | Acid. | 1023 | 49·6144 | 2·5682 | 0·4350 | 0·6149 | 31·9801 |
| 7 | 69600 | 830 | Acid. | 1026·5 | 52·6064 | 2·5625 | 0·4949 | 0·6536 | 34·2450 |
| 8 | 70000 | 930 | Acid. | 1025 | 56·5945 | 2·5102 | 0·5373 | 0·6738 | 31·5995 |

TABLE IV.—WITH AMMONIUM BROMIDE.

| May. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. | Amount of $(NH_4)Br$ taken, in grains. |
|---|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | gram. | gram. | grams. | |
| 9 | 69700 | 1040 c. c. | Acid. | 1024 | 59·5416 | 2·4514 | 0·4895 | 0·6630 | 34·2265 | 25 at 10 a. m.<br>25 3 p. m.<br>25 11 p. m. |
| 10 | 69700 | 1130 | Acid. | 1024·5 | 66·0864 | 2·2248 | 0·6581 | 0·6690 | 33·3503 | 33 at 10 a. m.<br>33 3 p. m.<br>34 11 p. m. |
| 11 | 70100 | 990 | Acid. | 1025 | 59·1092 | 2·5581 | 0·5470 | 0·6683 | 34·8876 | 40 at 2 p. m.<br>30 7 p. m.<br>30 11 p. m. |
| 12 | 70400 | 1130 | Acid. | 1024 | 64·7062 | 2·8177 | 0·6051 | 0·7051 | 36·1377 | 50 at 2 p. m.<br>50 7 p. m.<br>50 11 p. m. |

TABLE V.—WITHOUT BROMIDE.

| May. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | gram. | gram. | grams. |
| 13 | 70400 | 970 c. c. | Acid. | 1024·5 | 56·7291 | 2·3746 | 0·4329 | 0·6936 | 33·5846 |
| 14 | 70400 | 835 | Acid. | 1025·5 | 50·8766 | 2·1505 | 0·4081 | 0·7156 | 31·2645 |

However this may be, the use of ammonium bromide in our experiments gave rise to more unpleasant symptoms than the use of like amounts of the corresponding potassium salt. Like the potassium salt, ammonium bromide caused increased acidity in the urine and a brighter color. As to its influence on metabolism, a study of Table No. IV, and comparison with No. III, plainly shows a decided accelerating influence on the excretion of urea; the average of the results, moreover, shows a very slight diminution in the excretion of total phosphoric acid, at the same time it would appear, in accord with what was observed with potassium bromide, that the diminution was greatest with the smallest amounts of bromide, as on May 9th and 10th and on the 13th and 14th after withdrawal of the bromide. With the largest amount of ammonium bromide, on the other hand, phosphoric acid appeared to be increased in amount, thus according with what Dr. Bill observed with like quantities of potassium bromide.

The average difference in the two series of results is shown by the following table:

| | Average of Table No. III without $(NH_4)Br$. | Average of No. IV with $(NH_4)Br$. |
|---|---|---|
| Total quantity of urine | 915 c. c. | 1072 c. c. |
| Sp. Gr. | 1024, 8 | 1024, 4 |
| Total solid matters | 54·3970 grams. | 62·3608 grams. |
| Total $P_2O_5$ | 2·5643 | 2·5130 |
| $P_2O_5$ in combination with Ca and Mg | 0·4972 | 0·5749 |
| Uric acid | 0·6599 | 0·6751 |
| Urea | 32·8579 | 34·6505 |

It is thus seen that diuretic action is even greater with the ammonium salt than with potassium bromide; likewise the excretion of both urea and uric acid is greater under the influence of ammonium bromide than in the case of the potassium salt; as to phosphoric acid the table of averages shows practically nothing, but as before observed a study of the individual results does indicate some action of the salt, although diminution in the excretion of phosphoric acid under the influence of ammonium bromide cannot be so surely claimed as with the potassium salt.

After withdrawal of the ammonium bromide, the urine was examined for two days more; the results showing the same, or even greater drop in the excretion of urea, observed under like conditions with the potassium salt (Table No. V.). Hence, so far as our experiments extend, the influence of the two salts on the metabolism of the body is very much alike, differing only in extent of action; the ammonium

salt, as might be expected, causing the largest excretion of urea, not, however, necessarily from any greater influence on proteid metabolism, but merely as furnishing a certain amount of ammonia to be excreted as urea. Finally, our results, with both salts, fail to show that diminution in the excretion of phosphoric acid to be expected from active hypnotic agents, and in this respect, therefore, our results show nothing antagonistic to Dr. Bill's conclusion "that bromide of potassium in its legitimate action, is an anæsthetic to the nerves of the mucous membranes and a depressor of their action. Its hypnotic effects are secondary."

## XII.—Influence of Cinchonidine Sulphate on Metabolism. By R. H. Chittenden and Henry H. Whitehouse, Ph.B.

While much attention has been paid to the physiological study of the cinchona alkaloids; quinine particularly having been experimented with by several observers, to ascertain its influence on the metabolism of the body; we have not been able to find any recorded statements bearing on the action of the closely related alkaloid, cinchonidine. In physiological action, quinine is taken as a type of the group; cinchonine is stated* to be similar to quinine but less powerful, and that its history in the organism is parallel with that of quinine; cinchonidine is likewise stated to be weaker than quinine and in physiological action, apparently its equivalent when taken in doses one-third larger. Presumably, therefore, its action on the metabolism of the body is similar to that of quinine, although apparently no attempt has been made to determine this point. In view of the special action of quinine on nitrogenous metabolism, we have devoted our attention mainly to a study of the influence of cinchonidine on the excretion of urea, uric acid, phosphoric acid and chlorine. The experiments were conducted wholly on the person of one of us (H. H. W.) under uniform conditions of diet, exercise, etc. The diet, weighed out accurately each day, was composed of

255 grams meat (beef).
255 grams wheat bread.
149 grams potatoes.
50 grams oat meal.
35 grams butter.
21 grams sugar.
570 grams milk.
350 grams water.

This diet, divided into three definite portions each day, was taken for some time previous to the experiments, so that the system might become accustomed to it and the metabolism of the body brought to a constant point. The body weight was determined each morning; the urine collected from nine A. M. of one day to nine A. M. of the next, making the 24 hours' urine, the analysis being made the same

* H. C. Wood, Therapeutics, p. 81.

day. Urea was determined by Pflüger's * modification of Liebig's method, chlorine being removed by a standard solution of silver nitrate. Chlorine was determined by evaporating 10 c. c. of the urine with a weighed amount of potassium nitrate in a platinum crucible, igniting until the organic matter was completely removed, dissolving in water, acidifying with nitric acid, neutralizing with calcium carbonate and then titrating with silver nitrate solution. Uric acid was determined by Heintz's method as modified by Zablins† and phosphoric acid with a standard solution of uranium nitrate.‡ The amount of solid matter was calculated by the use of Christison's formula.

After taking the above diet for some time, the urine was analyzed for seven consecutive days prior to the exhibition of cinchonidine. The results, seen in Table No. I, show a very close agreement in the daily excretions.

On the 11th of May the first dose of cinchonidine sulphate was taken. The alkaloid salt was a finely crystallized preparation obtained from Powers and Weightman. The daily dose was usually divided into three portions and taken in tiny gelatine capsules, about five hours apart. In view of the fact that cinchonidine is a weaker alkaloid than quinine, it was not deemed necessary to try the influence of very small doses; on the first day, therefore, 15 grains of the salt were taken; on the second day 21·8 grains; on the third day 35·1 grains; and on the fourth 50 grains, making a total of 121·9 grains of cinchonidine sulphate in four consecutive days.§ The results of the analyses of the four days' urine, as well as those of the three following days, on which no cinchonidine was taken, are shown in Table No. II.

Comparing these results with those in Table No. I, and in Table No. III, it is seen that cinchonidine exercises a very decided influence on the nitrogenous metabolism of the body. Urea is at once affected: its excretion on the first day even, is diminished 6 per cent., while on the second day it is diminished 10 per cent., and on the fourth day when the largest dose of cinchonidine was taken, the excretion of urea was 16 per cent. less than in the normal urine. The influence

* Pflüger's Archiv, vol. xxi, p. 248.

† Die Lehre vom Harn, Salkowski and Leube, p. 94–95.

‡ Die Lehre vom Harn, p. 184.

§ While taking the larger doses of cinchonidine, an intense ringing in the ears was temporarily experienced (cinchonism) together with partial deafness and slight dizziness. On one or two occasions a slight nausea was felt.

TABLE I.—NORMAL URINE.

| May. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Chlorine. | Total $P_2O_5$. | Uric acid. | Urea. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | grams. | gram. | grams. |
| 4 | 59400 | 975 c. c. | Acid. | 1027·5 | 64·191 | 6·082 | 2·586 | 0·774 | 40·801 |
| 5 | 59400 | 950 | Acid. | 1028 | 63·713 | 5·553 | 3·044 | 0·797 | 42·684 |
| 6 | 59200 | 905 | Acid. | 1027 | 58·471 | 5·493 | 2·708 | 0·721 | 41·877 |
| 7 | 59600 | 990 | Acid. | 1027 | 63·962 | 6·510 | 2·710 | 0·819 | 42·999 |
| 8 | 59600 | 1077 | Acid. | 1026·5 | 68·262 | 6·324 | 2·810 | 0·768 | 41·464 |
| 9 | 59600 | 1055 | Acid. | 1026·5 | 66·867 | 6·166 | 3·219 | 0·749 | 41·640 |
| 10 | 59600 | 1052 | Acid. | 1026 | 65·887 | 6·149 | 3·040 | 0·764 | 41·985 |

TABLE II.—SHOWING THE EFFECTS OF CINCHONIDINE.

| May. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Chlorine. | Total $P_2O_5$. | Uric acid. | Urea. | Am't Cinchonidine sulphate taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | grams. | gram. | grams. | grains. |
| 11 | 59600 | 920 c. c. | Acid. | 1027 | 59·440 | 5·044 | 2·776 | 0·713 | 39·715 | 15 |
| 12 | 59900 | 975 | Acid. | 1026·5 | 61·797 | 5·886 | 2·693 | 0·779 | 37·967 | 21·8 |
| 13 | 60000 | 1195 | Acid. | 1024 | 68·428 | 7·435 | 2·529 | 0·792 | 38·322 | 35·1 |
| 14 | 60000 | 1022 | Acid. | 1024 | 58·522 | 5·855 | 2·128 | 0·575 | 35·115 | 50 |
| 15 | 60200 | 1030 | Acid. | 1023 | 56·467 | 6·725 | 1·795 | 0·598 | 35·481 | 0 |
| 16 | 59400 | 950 | Acid. | 1026·5 | 60·212 | 6·028 | 2·390 | 0·666 | 35·989 | 0 |
| 17 | 58800 | 975 | Acid. | 1027·5 | 64·191 | 5·586 | 2·801 | 0·711 | 39·856 | 0 |

TABLE III.—SHOWING THE EFFECTS OF CINCHONIDINE.

| May. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Chlorine. | Total $P_2O_5$. | Uric acid. | Urea. | Am't Cinchonidine sulphate taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | grams. | gram. | grams. | grains. |
| 18 | 59600 | 900 c. c. | Acid. | 1028·5 | 61·468 | 4·879 | 3·227 | 0·701 | 40·279 | 0 |
| 19 | 59600 | 920 | Acid. | 1028·5 | 62·834 | 5·270 | 3·019 | 0·744 | 43·453 | 0 |
| 20 | 59000 | 950 | Acid. | 1029·5 | 67·225 | 5·735 | 3·109 | 0·893 | 43·354 | 0 |
| 21 | 59000 | 875 | Acid. | 1030 | 62·997 | 4·851 | 3·085 | 0·699 | 40·999 | 50 |
| 22 | 59500 | 1047 | Acid. | 1027·8 | 69·704 | 6·708 | 2·674 | 0·705 | 40·407 | 45·8 |

TABLE IV.—SHOWING AVERAGE RESULTS.

| | Table I, Normal urine. | Table II, with Cinchonidine. | Table II, 3 days following the last dose of Cinchonidine. | Table III, without Cinchonidine. | Table III, with Cinchonidine. |
|---|---|---|---|---|---|
| Total quantity urine | 1001 c. c. | 1028 c. c. | 985 c. c. | 923 c. c. | 961 c. c. |
| Sp. Gr. | 1027 | 1025·4 | 1025·6 | 1028·8 | 1029 |
| Total solid matters | 64·408 grams. | 62·047 grams. | 60·290 grams. | 63·842 grams. | 66·351 grams. |
| Chlorine | 6·040 | 6·055 | 6·113 | 5·295 | 5·780 |
| Total $P_2O_5$ | 2·874 | 2·532 | 2·329 | 3·118 | 2·855 |
| Uric acid | 0·770 | 0·715 | 0·658 | 0·779 | 0·702 |
| Urea | 41·421 | 37·780 | 37·109 | 42·362 | 40·703 |

of cinchonidine on the elimination of urea continues to be felt after discontinuing the use of the alkaloid; thus even three days after the last dose of cinchonidine, the elimination of urea is 6 per cent. less than in the normal urine. On the fourth day it is nearly back to the normal amount [see Table No. III.], and on the fifth and sixth days the excretion of urea rises somewhat above the average. Uric acid does not appear to be correspondingly affected. It is only under the influence of the largest doses, or rather under the long continued action of the alkaloid that the excretion of uric acid is diminished; thus on May 14th, when the final dose of 50 grains of cinchonidine sulphate was taken, the excretion of uric acid was for the first time diminished; its diminution, however, was very perceptible and it continued for the two succeeding days, after withdrawal of the alkaloid salt. Phosphoric acid was greatly diminished in amount under the influence of cinchonidine; the diminution commencing to show, as in the case of urea, with the first dose of the alkaloid salt, then gradually increasing in amount with increase in the dose of cinchonidine until maximum diminution was reached on the day after the final dose of the alkaloid. The alkaloid salt, moreover, appears to have had a slight diuretic action.

On the 21st of May, cinchonidine sulphate was again taken, the results of the analyses of the 19th and 20th showing that the urine had returned to its normal composition. Accordingly, 95·8 grains of the salt were taken on the 21st and 22d, producing the same results (Table No. III.) as noticed in the first series, viz: diminution in the amount of urea and uric acid excreted, a like diminution in the amount of phosphoric acid and an increase in the total amount of fluid.

Table No. IV. shows the *average* results under the different conditions of the experiments.

It is evident then, that cinchonidine has the power of lessening very materially the elimination of nitrogen, i. e. the consumption of tissue.

Ranke* first pointed out the retarding influence of quinine on the elimination of uric acid. Zuntz found by experiment upon himself that 25 grains of quinine reduced his elimination of urea nearly 40 per cent. A like diminution in the excretion of urea, under the influence of quinine, was noticed by Rabuteau in experiments upon dogs and also by Hermann von Boeck.† The most interesting experiments, however, with quinine are those carried out by Dr. G.

* Quoted from Dr. H. C. Wood, Therapeutics, p. 74.

† Zeitschrift für Biologie, vol. vii, p. 422.

Kerner,* and more recently by Dr. Prior† and Dr. Sassetzky.‡ In Dr. Prior's article is to be found a very complete account of the literature of the subject. Dr. Kerner found that taking 9·3 grains of quinine hydrochloride per day in divided doses, for 3 days, making a total of 27·9 grains, caused a diminution in the excretion of urea, amounting on an average for the three days to 12 per cent., while the excretion of uric acid under like conditions was diminished 54 per cent. Phosphoric acid, too, was diminished somewhat; on an average about 4 per cent. per day. Diuretic action was very slight.

With larger doses of quinine; 77·5 grains of the hydrochloride in divided doses during three days; diuretic action was quite pronounced, the average increase in volume, amounting to 200 c.c. Moreover, urea was diminished on an average 23 per cent. per day, uric acid 82 per cent. and phosphoric acid 15 per cent. Oppenheim,§ however, by a dose of 30·8 grains of quinine found an increase in the daily excretion of urea amounting to 4 grams, and he considers that Kerner's results are due to diminution of digestive action. It is certain that both quinine and cinchonidine do interfere with the proteolytic action of the gastric and pancreatic juices,‖ but this retarding action can hardly be taken as explaining in full, the results obtained by Kerner or those obtained by us with cinchonidine.

Prior, moreover, by very carefully conducted experiments with quinine, has completely corroborated Kerner's results and has shown in addition, by a daily determination of nitrogen in the fæces, that diminution of urea and uric acid is not due to lack of digestive action, as suggested by Oppenheim. Prior's results show on an average, without reference to the size of the dose, the following effects of the quinine.¶

| Quantity of urine. | Urea. | Uric acid. | Sodium chloride. | Sulphuric acid. | Phosphoric acid. |
|---|---|---|---|---|---|
| increase | decrease | decrease | decrease | decrease | decrease |
| 10·65 per cent. | 19·60 % | 72·29 % | 9·06 % | 33·70 % | 23·38 % |

Sassetzky's results with fever patients, also corroborate Kerner's statements.

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* Pflüger's Archiv, vol. iii, p. 104.

† Ueber den Einfluss des Chinin auf den Stoffwechsel des gesunden Organismus. Pflüger's Archiv, vol. xxxiv, p. 237.

‡ Ueber den Einfluss fieberhafte Zustände und Antipyretischer Behandlung auf den Umsatz der stickstoffhaltigen Substanzen und die Assimilation stickstoff-haltiger Bestandtheile der Milch. Virchow's Archiv, vol. xciv, p. 485.

§ Pflüger's Archiv, vol. xxiii, p. 476–477.

‖ Chittenden and Allen; Chittenden and Cummins. Trans. Conn. Acad., vol. vii.

¶ Pflüger's Archiv, vol. xxxiv, p. 263.

v. Boeck considers that quinine owes its retarding influence on proteid metabolism to a direct action of the alkaloid upon the cells and their activity, although the alkaloid doubtless does unite with albumin or alter its constitution so as to render it less readily decomposable. Metallic salts, as lead and mercury, certainly form compounds with albumin difficultly decomposable, as also does arsenic, and v. Boeck suggests that these metallic poisons unite with the proteid matter of the various organs of the body, while quinine unites simply with the circulating albumin, explaining in this manner the ready elimination of quinine as compared with the slow excretion of mercury or arsenic, the latter of which v. Boeck* has shown has little if any influence on proteid metabolism. Prior, moreover, states that diminution in the urine of the end-products of nitrogenous metabolism is due, not to hindering of their excretion, but to actual hindering of their formation.

Comparing now Kerner's results, with the results obtained by us with cinchonidine, we see great similarity of action but decided difference in extent, particularly so far as the excretion of uric acid is concerned. With cinchonidine, the greatest average daily diminution in uric acid amounts to but 15 per cent., and this after taking about 121 grains of the alkaloid during four consecutive days. Selecting the lowest single result, that obtained on the day 50 grains of cinchonidine were taken and comparing the diminution then, with the average normal excretion, it is seen to amount to but 26 per cent. In the case of urea and phosphoric acid, the divergence is not so great; thus for urea the average daily diminution was 11 per cent. for the three days following the last dose of cinchonidine, while the greatest diminution noticed in any one day was 16 per cent.; with phosphoric acid the average diminution for the same period amounted to 19 per cent., while the greatest diminution noticed any one day was 38 per cent.; a diminution which at no time was reached in Kerner's experiments with quinine.

Thus in drawing a comparison between Kerner's results with 77·5 grains of quinine distributed through three days, and our results with 121 grains of cinchonidine extended over four days, we see two striking points of difference; with quinine there is a diminution in the amount of uric acid excreted of 82 per cent., with cinchonidine an average diminution of but 15 per cent.; with quinine there is a diminution of phosphoric acid amounting to 15 per cent., with cinchonidine, on the other hand, a diminution of 19 per cent. Hence it is to

* Zeitschrift für Biologie, vol. vii, p. 430.

be seen that cinchonidine has a far less pronounced specific action on the excretion of uric acid than quinine, while on the other hand, diminution of phosphoric acid is much more pronounced with cinchonidine than with quinine. In Prior's experiments, however, with quinine, diminution of phosphoric acid is more pronounced.

Is this diminution in the excretion of phosphoric acid under the influence of cinchonidine to be attributed simply to decrease of proteid metabolism, or is it in part due to a special action of cinchonidine on the metabolism of some phosphorized principles, presumably those of nerve tissue? If due to general decrease of proteid metabolism, we might expect to find that the addition of any non-nitrogenous principle to our fixed diet, whereby the decomposition of albuminous matter would be diminished, would cause a corresponding decrease in the excretion of phosphoric acid, or in other words that diminution of urea and phosphoric acid excreted, would be in the same ratio as noticed under the influence of cinchonidine.

This question we have endeavored to answer by a study of the influence of pure glucose on the elimination of urea, uric acid, and phosphoric acid, under the same conditions of diet etc., as observed in the experiments with cinchonidine.

The influence of carbohydrate food on proteid metabolism has been illustrated in many ways by various investigators, but so far as we know, no experiments with pure glucose have ever been tried. Through the courtesy of Dr. Arno Behr, of Chicago, we have been supplied with an abundance of chemically pure anhydrous glucose, which we have used in the following experiment. Before taking the sugar, the urine was analyzed for ten consecutive days, to insure an accurate average of the normal excretion under the conditions of the experiment. The results are shown in Table No. V.

200 grams of glucose were then taken daily in addition to the fixed diet, for nine consecutive days. The effect on the excretion of urea, etc., is shown in Table No. VI. At no time was sugar to be detected in the urine by Trommer's test. A comparison of the two tables shows the usual effects of carbohydrate matter on the excretion of nitrogen, viz: a diminution in the amount of both urea and uric acid. The volume of the fluid excreted, appears to be considerably lessened by taking the glucose. The excretion of phosphoric acid is likewise diminished.

TABLE V.—NORMAL URINE.

| Day of month. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Chlorine. | Total $P_2O_5$. | Uric acid. | Urea. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | grams. | grams. | grams. |
| 1 | 59400 | 1045 c. c. | Acid. | 1029 | 72·658 | 6·636 | 3·220 | 1·060 | 46·284 |
| 2 | 59600 | 1060 | Acid. | 1028·5 | 72·395 | 7·030 | 3·249 | 1·068 | 46·463 |
| 3 | 59400 | 1070 | Acid. | 1028 | 71·761 | 6·194 | 3·333 | 0·802 | 46·607 |
| 4 | 59200 | 1042 | Acid. | 1029 | 72·450 | 6·090 | 3·167 | 0·907 | 45·770 |
| 5 | 60000 | 960 | Acid. | 1028 | 64·384 | 6·258 | 2·886 | 0·928 | 43·161 |
| 6 | 59800 | 990 | Acid. | 1028·5 | 67·615 | 6·399 | 2·659 | 0·804 | 44·078 |
| 7 | 59800 | 1004 | Acid. | 1026 | 62·404 | 6·320 | 2·621 | 0·722 | 44·000 |
| 8 | 59800 | 1090 | Acid. | 1027 | 70·423 | 7·780 | 3·304 | 0·715 | 47·103 |
| 9 | 59400 | 1073 | Acid. | 1027·5 | 70·643 | 8·141 | 3·045 | 0·921 | 45·900 |
| 10 | 59400 | 970 | Acid. | 1028 | 65·045 | 6·978 | 2·597 | .... | 45·890 |

| Day of month. | Body weight. | Total quantity Urine. | Reaction. | Sp. Gr. | Total solid matters. | Chlorine. | Total $P_2O_5$. | Uric acid. | Urea. | Am't of Glucose taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| | grams. | | | | grams. | grams. | grams. | gram. | grams. | grams. |
| 11 | 59600 | 985 c. c. | Acid. | 1028·5 | 63·858 | 7·200 | 2·700 | 0·664 | 43·588 | 200 |
| 12 | 59500 | 900 | Acid. | 1030 | 64·797 | 6·727 | 2·781 | 0·721 | 42·114 | 200 |
| 13 | 59300 | 865 | Acid. | 1029 | 60·143 | 6·028 | 2·731 | 0·693 | 40·778 | 200 |
| 14 | 59800 | 840 | Acid. | 1028·5 | 57·370 | 4·862 | 2·440 | 0·663 | 40·009 | 200 |
| 15 | 59900 | 970 | Acid. | 1028·5 | 66·249 | 6·433 | 2·916 | 0·795 | 40·070 | 200 |
| 16 | 60000 | 1047 | Acid. | 1028 | 70·219 | 7·120 | 3·130 | 0·850 | 42·502 | 200 |
| 17 | 59600 | 950 | Acid. | 1028 | 63·713 | 6·674 | 2·832 | 0·642 | 40·887 | 200 |
| 18 | 59800 | 970 | Acid. | 1028 | 65·055 | 6·106 | 2·654 | 0·787 | 39·800 | 200 |
| 19 | 60000 | 965 | Acid. | 1026·5 | 61·163 | 5·640 | 2·527 | 0·684 | 38·752 | 200 |
| 20 | 60200 | 1072 | Acid. | 1026 | 66·630 | 7·290 | 2·934 | 0·846 | 41·740 | 0 |
| 21 | 59600 | 1014 | Acid. | 1026 | 63·025 | 6·553 | 2·655 | 0·809 | 39·835 | 0 |
| 22 | 60000 | 1076 | Acid. | 1026 | 66·879 | 6·289 | 3·108 | 0·901 | 45·439 | 0 |
| 23 | 59000 | 1056 | Acid, | 1026 | 65·636 | 6·350 | 2·962 | 0·891 | 44·492 | 0 |

These points of difference are all shown more clearly in the following table of averages :

| | Average of | |
|---|---|---|
| | Normal urine. | Urine under the influence of glucose. |
| Total quantity urine | 1030 c. c. | 938 c. c. |
| Sp. gr. | 1027·9 | 1028 |
| Total solid matters | 68·97 grams. | 63·62 grams. |
| Chlorine | 6·78 | 6·31 |
| Total $P_2O_5$ | 3·00 | 2·75 |
| Uric acid | 0·88 | 0·72 |
| Urea | 45·47 | 40·94 |

Coming now to the point at issue, viz : the relation between the amounts of urea and phosphoric acid excreted, we find that under the influence of glucose the average diminution of urea amounts to 10 per cent., while the average diminution of phosphoric acid under the same conditions is 8·34 per cent.

With cinchonidine, on the other hand, the average diminution of urea amounts to but 8·8 per cent., while the average diminution of phosphoric acid under like conditions is 11·9 per cent. Or, if we take the average of the three days following the last dose of cinchonidine, when both urea and phosphoric acid reach their maximum diminution, and compare these results with the average of the normal excretion we see that while the diminution of urea amounts to 10·4 per cent., the average diminution of phosphoric acid is raised to 18·97 per cent. Consequently, it would appear that while cinchonidine lowers the rate of decomposition of proteid matter in the body, it also has an effect upon the decomposition of some phosphorized principles, that being the only plausible explanation of the increased diminution of phosphoric acid noticed under the influence of the cinchonidine salt.

## XIII.—The Post-mortem Formation of Sugar in the Liver, in the presence of Peptones. By R. H. Chittenden and Alexander Lambert, B.A., Ph.B.

Claude Bernard's discovery in 1848, that the liver contains sugar, both before and after death, led at once to the inquiry as to the source of the sugar. This was apparently answered by Bernard's later discovery of glycogen, an amylaceous body readily convertible into sugar by acids and various ferments. Thus, Bernard's theory that the liver sugar resulted exclusively from glycogen has long been an accepted fact. In 1880, however, Seegen and Kratschmer in the first of a series of investigations,* state that the sugar formed in the liver does not have its origin, as supposed by Bernard, *wholly* in glycogen but that it is undoubtedly formed *in part* from other material. In a later communication† the same investigators show, in corroboration of their previous statement, 1. that the amount of sugar in the liver is increased very rapidly after death, in one case nearly 50 per cent. of the entire amount being formed within 10 minutes, while the whole process comes to an end inside of 24 hours; 2. that the glycogen formed in the liver is much more resistant to ferment action than has hitherto been supposed and that consequently the post-mortem formation of sugar by the action of a ferment upon glycogen could not take place so rapidly as the above. Moreover, direct experiments with dogs and with rabbits showed that in the first few hours after death, there was but little if any diminution in the amount of glycogen. Hence, Seegen and Kratschmer claim that the amount of glycogen remaining essentially the same, while the amount of sugar is greatly increased, tends to show conclusively that the liver sugar must be formed from some other material than glycogen and they venture the opinion that this source, whatever it may be, furnishes all of the liver sugar.

Boehm and Hoffmann,‡ however, take exception to the views of Seegen and Kratschmer, claiming possible analytical inaccuracies from the methods of procedure. They show, moreover, by experi-

* Ueber Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxii, p. 236.

† Pflüger's Archiv, vol. xxiv, p. 467.

‡ Ueber die postmortale Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxiii, p. 205.

ments on cats and dogs, that after death, contrary to the statements of Seegen, increased formation of sugar is attended with a corresponding decrease of glycogen, at least within such limits as are incident to the errors of experiment; further that in the case of a cat's liver 32 per cent. of the liver glycogen disappeared in 24 hours after death, thus indicating less resistance to the action of ferments than would be implied by Seegen's and Kratschmer's results.

In a later investigation,* Seegen shows that pieces of finely divided liver, kept in contact for an hour or longer with a solution of peptone yield a larger amount of sugar and even of total carbohydrates, than equal weights of the same liver under like conditions of treatment, without peptones. These results were obtained with the livers of calves, rabbits and dogs. Seegen, therefore, concludes that the liver is capable of forming from peptones, sugar and carbohydrates which are convertible into sugar.

A study of the analytical data plainly shows that the increase in sugar and total carbohydrates in the presence of peptone, although pronounced, is not great. The following experiment† with a calf's liver obtained from the market shows the most marked increase.

| | | With peptone. | | Without peptone. | |
|---|---|---|---|---|---|
| No. | Time of the experiment. | Sugar. | Total carbohydrates. | Sugar. | Total carbohydrates. |
| I. | 30 minutes | 3·84 % | 9·52 % | 3·40 % | 8·8 % |
| II. | 48 hours | 3·56 | 8·92 | 3·70 | 8·6 |
| III. | 96 " | 2·66 | 8·00 | 2·82 | 7·8 |

Here the increase in total carbohydrates is seen to be only 0·72 per cent. and of sugar only 0·44 per cent. after 30 minutes. In Nos. II and III, longer standing in contact with the peptone tends to reduce the amount of sugar and to diminish the increase of total carbohydrates. This is attended with increase of acidity and Seegen considers that a portion of the sugar is decomposed in this long contact with peptone with formation of acid.

In a still later communication,‡ Seegen reports the results of other experiments tending to confirm his theory of the formation of carbohydrate matter from peptones in the liver. Thus, by feeding peptones to dogs, Seegen found that the content of sugar in the livers of eight dogs was considerably greater that in the normal liver, taking for the latter value the average of a number of determinations.

* Die Einwirkung der Leber auf Pepton. Pflüger's Archiv, vol. xxv, p. 165.

† Pflüger's Archiv, vol. xxv, p. 171.

‡ Pepton als Material für Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxviii, p. 99.

Likewise, by the injection of peptone solutions directly into the portal circulation of dogs, Seegen found the amount of sugar in the liver increased two and even nearly three times above the normal amount. Lastly, by warming portions of freshly excised liver at 40° C., with a solution of peptone in water and some fresh, defibrinated blood, through which a constant current of air was made to pass, the amount of both sugar and total carbohydrates was considerably greater than under like conditions, but without peptones. The following experiment* taken from Seegen's account, illustrates the average increase of carbohydrates under this method of treatment.

Two portions of a dog's liver taken 15 minutes after death, were mixed with 50 c. c. of water and 50 c. c. of defibrinated blood. To one portion 5 grams of peptone were added and air passed through the mixture for 5 hours. Following are the results obtained in both:

| Wt. of portion of liver. | Method of treatment. | Liver sugar. | Total carbohydrates. | Glycogen. |
|---|---|---|---|---|
| 40 grams. | without peptone and blood, | 3·04 % | 6·9 % | 2·12 % |
| 40 " | with peptone and blood, | 3·87 | 8·4 | 2·02 |

Other experiments indicated that peptones themselves are without diastatic action and that the blood and air (to form oxyhaemoglobin) are by themselves without influence on the liver. Hence Seegen concludes that the liver cells, retained in a living condition by the action of blood rendered arterial by a current of air, are capable of forming from peptone more or less sugar; thus establishing, if true, that the animal organism is able to form carbohydrates from albuminous material.

This is certainly a very important question, for if Seegen's views are correct they overthrow the long accepted belief in the origin of liver sugar in the hepatic glycogen. It is true that Bernard himself, before his discovery of glycogen, thought that the liver sugar originated in albumin and there have always been, up to the present time, difficulties in explaining the origin of liver carbohydrates on the dehydration theory alone. As is well known, a certain amount of glycogen is formed during a purely animal diet and in chronic cases of Diabetes the excretion of sugar is continued even on a pure albuminious diet. Moreover, the suggestion has been before made that peptones in their passage through the liver undergo change. Thus Plosz and Gyergyai† noticed that while considerable peptone was to be found in the blood of the mesenteric veins and more or less in

* Pflüger's Archiv, vol. xxviii, p. 123.

† Ueber Peptone und Ernahrung mit denselben. Pflüger's Archiv, vol. x, p. 536.

the liver, only the merest trace was to be found in the blood of the hepatic vein, indicating thereby a decomposition of peptone in its passage through the liver.

Maydl* claims that since the products of the decomposition of all forms of glycogen are the same, it follows that the glycogens themselves are all identical, and since it is extremely improbable that the various carbohydrates with their different chemical constitutions should give *one* glycogen, he argues that it all must come from one source, viz: albumin.

This is not the place, however, to discuss the relative merits of the dehydration and storage theories, it is enough simply to understand that the possible origin of liver sugar in proteid matter is one which would make clear many hitherto unexplained points. The great obstacle, has been to understand where and in what manner the liver sugar could be so formed. Seegen's views therefore are of great importance, and are, moreover, in no sense, wholly inconsistent with previous ideas, but the question at once suggests itself whether the analytical data on which they are founded are sufficient to warrant their adoption.

The determination of sugar in organic fluids is not without difficulty, and where slight variations in results may cause differences of half a per cent. or more, it becomes an extremely delicate matter to determine how far such results shall be trusted. Consequently, whatever may be said as to whether the formation of sugar in the manner indicated by Seegen is a natural or an artificial process, we need first of all to know positively whether the liver under any circumstances is able to form sugar or other carbohydrate matter from peptones. This all hinges on the accuracy of Seegen's results, obtained by warming portions of liver with peptones. If an increase of sugar and total carbohydrates is found in the presence of peptone, then we must conclude that the latter has at least some influence on the formation of the liver sugar. Recent experiments† have plainly shown that neutral peptone has a stimulating influence on the amylolytic action of ptyalin of saliva and diastase of malt; both of these ferments convert more starch into sugar in the presence of peptone than without and it is natural to suppose that the presence of peptone would similarly affect the amylolytic ferment which presumably acts upon glycogen. Seegen's results, however, appear to show that while sugar is increased

* Zeitschrift für physiol. Chem., vol. iii, p. 196. Ueber die Abstammung des Glykogens.

† Trans. Conn. Acad., vol. vi, p. 343, vol. vii, p. 44.

in the presence of peptone, glycogen remains nearly stationary, or if diminished, not at all in proportion to the increase in sugar. Boehm and Hoffmann, however, found the liver glycogen much less resistant and that its decrease was in proportion to the increase in sugar. Delprat,* too, came to similar conclusions and could obtain no proof whatever, of the correctness of the views advanced by Seegen and Kratschmer. We have, therefore, in view of the importance of the subject, undertaken a study of the question in the hopes of throwing some additional light upon the matter. In this, however, we have limited ourselves entirely to a study of the post-mortem formation of sugar and carbohydrates by the liver in the presence of peptones.

### *Methods employed.*

The animals experimented with, mainly rabbits, were killed by severing the jugular vein, the blood being collected and defibrinated. The liver was quickly taken out, the gall bladder removed and the liver then converted into a fine pulp by chopping, since it is probable, as v. Wittich has suggested, that glycogen is unequally distributed through the liver. Two equal portions of the sampled and finely divided liver were accurately weighed out and placed in separate flasks; one, with a solution of peptone and a known volume of blood, the other with an amount of distilled water equal in volume to that of the two former. Both were then placed in a bath and warmed at 38–40° C. for the time of the experiment. A continuous current of air was made to pass through the blood solution in order to render it arterial. At the end of the experiment, the mixtures were poured into boiling water and extracted as long as a trace of glycogen could be detected in the fluids, by the iodine test. This usually took about two days, working on an average with 40 grams of liver. At the beginning of the extraction, the tissue was generally boiled with 400–500 c. c. of water for about fifteen minutes and then filtered through a funnel plugged with absorbent cotton. By repeating this operation four or five times, the greater portion of glycogen could be removed, but a complete extraction could be obtained only by long continued boiling with fresh quantities of water or long heating on the water-bath, the tissue being ground up occasionally in a suitable mortar. The various filtrates were evaporated on a water-bath and finally united and made up exactly to 500 c. c., after which the extracts were filtered through dry paper filters to

* Jahresbericht für Thierchemie, 1881, p. 321.

remove any traces of suspended matter which might have passed the cotton. Of these fluids, 200 c. c. of each were used for the determination of glycogen and sugar, and 200 c. c. also, for the determination of total carbohydrates.

*Determination of glycogen and sugar.*—The 200 c. c. of fluid for the determination of glycogen and sugar were evaporated to a small bulk and then, when cool, precipitated by a large volume of alcohol. After standing 24 hours the clear supernatant fluid was filtered from the precipitated glycogen and peptones. The alcoholic filtrate and washings, containing the sugar, were then evaporated, the residue dissolved in water and made up to 100 c. c., in an aliquot portion of which the sugar was determined gravimetrically, by Allihn's* improved method.

The precipitate of glycogen, with its frequent admixture of peptone, was dissolved in water, the solution made up to 200 c. c. and then sufficient 10 per cent. hydrochloric acid added to make the solution contain 2 per cent. HCl. The mixture was then heated in a closed flask at 100° C. for 17 hours in order to convert the glycogen into dextrose, after which the solution was neutralized, concentrated somewhat, again made up to 200 c. c. and in an aliquot portion of this fluid, dextrose was determined by Allihn's method, from which was calculated the amount and percentage of glycogen. Delpratt† states that in attempting to determine glycogen by Brücke's method he found the results considerably higher than when the isolated glycogen was converted into sugar by boiling with acid and the glycogen calculated from the data obtained. In our own experiments, the frequent presence of peptone prevented entirely the use of Brücke's method. 12 hours heating at 100° C., however, with 2 per cent. hydrochloric acid was found in our case insufficient to completely convert the glycogen into dextrose, while 17 hours was found amply sufficient for complete conversion and at the same time allowed no decomposition of the sugar formed. This is well illustrated by the following experiments:

A. 0·7665 gram pure, dried glycogen dissolved in 100 c. c. of water, was heated at 100° C. for 12 hours with sufficient hydrochloric acid to make the entire fluid contain exactly 2 per cent. The solution was neutralized, care being taken that the reaction did not become alkaline, then concentrated and finally made up to 50 c. c.

14 c. c. gave 0·4215 gram Cu=0·2251 gram dextrose=0·2025 gram glycogen.
14 " " 0·4233 " Cu=0·2263 " " =0·2036 "

* Zeitschrift für analytische Chemie, xxii, p. 448.

† Jahresbericht für Thierchemie, 1881, p. 322.

The 14 c. c. should have contained 0·2414 gram dextrose, the equivalent of 0·2146 gram of glycogen.

B. 0·6190 gram glycogen dissolved in 100 c. c. of water was heated at 100° C. for 17 hours in the presence of 2 per cent. of hydrochloric acid. Solution was then neutralized, evaporated and made up to 50 c.c.

18 c. c. gave 0·4585 gram Cu=0·2470 gram dextrose=0·2223 gram glycogen.
18 " " 0·4555 " Cu=0·2454 " =0·2208 "

The 18 c. c. should have contained 0·2475 gram dextrose, equal to 0·2228 gram of glycogen. Hence, it is seen that 17 hours heating at 100° C. is needed for a complete conversion of glycogen into dextrose, which was the time invariably employed in the after experiments.

*Influence of peptone on the conversion of glycogen into sugar by* 2 *per cent. HCl at* 100° *C.*—The question naturally suggested itself in this connection whether the presence of peptone would interfere in any way with the complete conversion of glycogen into dextrose or whether the peptones by this long heating at 100° C. with the acid, would undergo any change by which reducing bodies might be formed and thus endanger the accuracy of the results. The latter point was tested by heating 2 grams of peptones in 100 c. c. of water containing 2 per cent. of hydrochloric acid for 17 hours at 100° C., at the end of which time no reduction at all could be obtained with Fehling's solution.

The first point was tested by the following experiment:

0·9290 gram of pure glycogen was dissolved in 100 c. c. of water, then 2 grams of peptone were added and sufficient acid for the solution to contain exactly 2 per cent. HCl, after which the mixture was heated at 100° C. for 17 hours. The solution was then neutralized, brought to a volume of 100 c. c. and the sugar determined.

10 c. c. gave 0·1985 gram Cu=0·1017 gram dextrose=0·0915 gram glycogen.
10 " " 0·2025 " Cu=0·1039 " =0·0934 "

whereas in the 10 c. c. then should be present, according to calculation 0·1032 gram dextrose, the equivalent of 0·0929 gram of glycogen. Consequently the presence of peptone does not interfere with the accurate determination of glycogen by this method.

*Influence of the presence of peptone on the determination of sugar by Allihn's method.*—Seegen* finds that the volumetric determination of sugar with Fehling's solution is not materially affected by the presence of peptone. By repeated experiments we have convinced ourselves, that in the use of the gravimetric method, the

* Pflüger's Archiv, vol. xxviii, p. 115.

presence of peptone may, unless certain precautions are taken, interfere slightly with exact determinations. With the Allihn method, variations of 2–5 milligrams in the amount of reduced copper are liable to occur if care is not taken in regulating the length of time the alkaline copper solution is heated after addition of the sugar solution. Under ordinary circumstances results most nearly in accord with theory are obtained by adding the sugar solution, as recommended by Allihn, to the previously heated Fehling's solution and then heating further until bubbles just begin to break upon the surface of the liquid. If heated longer, even only half a minute, a slight increase in the amount of reduced copper will generally be observed. Now whenever peptone is present to any extent in the sugar solution, we have found by experience that complete reduction does not take place quite so rapidly; the loss is not great, sometimes but a milligram or so, still the difference is appreciable. This, however, can be avoided by simply allowing the standard copper solution to boil for about 45 seconds after the addition of the sugar solution. Under such conditions, repeated trials have shown us, that the presence of peptone does not offer the slightest obstacle to accurate determinations of dextrose. Whenever, therefore, in the following experiments the solution to be tested contained peptone, the above rule has been invariably followed.

*Determination of total carbohydrates.*—For this purpose 200 c. c. of the liver extract were heated in a closed flask at 100° C. with sufficient 10 per cent. hydrochloric acid to ensure a content of 2 per cent HCl, for 17 hours. The solution was then nearly neutralized, care being taken that the fluid did not become alkaline, concentrated and finally brought to a volume of 200 c. c., in an aliquot portion of which the total carbohydrates in the form of dextrose were determined in the usual manner. Seegen* states that in the determination of total carbohydrates, the fluid, after heating with acid, always became very dark, which occasionally interfered somewhat with the determination of sugar. Delprat,† however, states that in his experiments the solution, under like conditions, became brownish yellow and generally deposited a flocculent brownish black precipitate of organic matter. Moreover, in some cases, particularly with the livers of dogs, cats and calves, the cuprous oxide, in determining total carbohydrates, would remain dissolved to a great extent, thus interfering with the accuracy of the volumetric determination, some-

* Pflüger's Archiv, vol. xxviii, p. 121.

† Jahresbericht für Thierchemie, 1881, p. 323–324.

times to the extent of even 1–2 c. c. of the sugar solution. In our experiments the acid solution was usually yellow or yellowish brown, and invariably at the end of the 17 hours contained the flocculent precipitate described by Delprat. By nearly neutralizing the solution, the amount of this precipitate was considerably increased and on then filtering the fluid, after having made it up to a volume of 200 c. c., considerable organic matter was removed. This, we found, had a decided influence on the accuracy of the determination, since alkaline solutions of this neutralization precipitate appeared to decidedly retard separation of the cuprous oxide. By paying attention to this point, we had no difficulty in obtaining fairly concordant results, by the use of the Allihn gravimetric method.

### *Experiment* I.

A large sized rabbit was killed, the blood collected and defibrinated, the liver quickly removed and finely chopped. Two portions of 40 grams each were weighed out and treated as follows :

| *A.* | *B.* |
|---|---|
| 40 grams liver. | 40 grams liver. |
| 50 c. c. of a 10 % solution of peptone. | 95 c. c. of water. |
| 25 c. c. of blood. | |
| 20 c. c. of water. | |

These were placed in flasks, and warmed at 40° C. for two hours. The liver was in contact with the peptone 40 minutes after the death of the animal. A continuous current of air was kept passing through *A*. Following are the analytical results:

Glycogen; total volume of the resultant sugar solution 200 c. c.
Sugar; total volume of the solution 100 c. c.
Total carbohydrates; volume of the resultant solution 200 c. c.

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amt.* | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·2345 gram. | 0·1209 gram. | 0·1088 gram. | 0·8704 gram. | 5·44 |
| 25 | 0·2360 | 0·1217 | 0·1095 | 0·8760 | 5·47 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·2675 gram. | 0·1386 gram. | 0·1247 gram. | 0·9976 gram. | 6·23 |
| 25 | 0·2659 | 0·1377 | 0·1239 | 0·9912 | 6·19 |
| | | *Sugar A.* | | | |
| 25 c. c. | 0·2260 gram. | 0·1164 gram. | ----- | 0·4656 gram. | 2·91 |
| 25 | 0·2255 | 0·1163 | ----- | 0·4652 | 2·90 |

* Total amount of glycogen, dextrose or carbohydrates calculated as dextrose, contained in the above volume (100 or 200 c. c.) and representing, therefore, the amount contained in two-fifths of the 40 grams of liver.

| Volume used. | Weight Cu. | Equivalent in dextrose. | Total amt. | Per cent. |
|---|---|---|---|---|
| | | *Sugar B.* | | |
| 25 c. c. | 0·2135 gram. | 0·1097 gram. | 0·4388 gram. | 2·74 |
| | | *Total Carbohydrates A.* | | |
| 12·5 c. c. | 0·2160 gram. | 0·1111 gram. | 1·7776 grams. | 11·10 |
| 20 | 0·3367 | 0·1768 | 1·7680 | 11·05 |
| | | *Total Carbohydrates B.* | | |
| 25 c. c. | 0·4030 gram. | 0·2146 gram. | 1·7168 grams. | 10·73 |
| 25 | 0·4042 | 0·2153 | 1·7224 | 10·76 |

The following table shows the average percentage results:

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams. | With peptones and blood (A), | 5·46 % | 2·91 % | 11·08 % |
| 40 | Without peptones and blood (B), | 6·21 | 2·74 | 10·75 |
| | | —0·75 | +0·17 | +0·33 |

From this it is seen that while in the presence of peptone and blood there is a slight increase of both total carbohydrates and sugar, there is also a more than corresponding decrease in the percentage of glycogen.

## *Experiment* II.

Liver of a rabbit, removed directly after death and treated in the same manner as in Experiment I.

| *A.* | *B.* |
|---|---|
| 40 grams sampled liver. | 40 grams sampled liver. |
| 50 c. c. of a 10 per cent. peptone solution. | 145 c. c. of water. |
| 25 grams blood. | |
| 70 c. c. of water. | |

Warmed 2 hours at 40° C., with a current of air passing through *A*.

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·3170 gram. | 0·1659 gram. | 0·1493 gram. | 1·1944 grams. | 7·46 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·3420 gram. | 0·1798 gram. | 0·1618 gram. | 1·2944 grams. | 8·09 |
| | | *Sugar A.* | | | |
| 25 c. c. | 0·2520 gram. | 0·1303 gram. | ----- | 0·5212 gram. | 3·26 |
| | | *Sugar B.* | | | |
| 10 c. c. | 0·0865 gram. | 0·0441 gram. | ----- | 0·4410 gram. | 2·75 |
| | | *Total carbohydrates A.* | | | |
| 10 c. c. | 0·2205 gram. | 0·1134 gram. | ----- | 2·2680 grams. | 14·15 |
| | | *Total carbohydrates B.* | | | |
| 10 c. c. | 0·2110 gram. | 0·1084 gram. | ----- | 2·1680 grams. | 13·55 |

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams. | With peptones and blood (A), | 7·46 % | 3·26 % | 14·15 % |
| 40 | Without peptones and blood (B), | 8·09 | 2·75 | 13·55 |
| | | —0·63 | +0·51 | +0·60 |

## *Experiment* III.

A small rabbit, treated in the same manner as the preceding:

| *A.* | *B.* |
|---|---|
| 25 grams sampled liver. | 25 grams sampled liver. |
| 50 c. c. of a 10 per cent. peptone solution. | 125 c. c. of water. |
| 50 grams of blood. | |
| 25 c. c. of water. | |

Warmed for 2 hours at 40° C., with a constant current of air passing through *A*.

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·0435 gram. | 0·0226 gram. | 0·0203 gram. | 0·1624 gram. | 1·62 |
| 25 | 0·0455 | 0·0236 | 0·0212 | 0·1696 | 1·69 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·0425 gram. | 0·0221 gram. | 0·0198 gram. | 0·1584 gram. | 1·58 |
| 25 | 0·0405 | 0·0211 | 0·0189 | 0·1512 | 1·51 |
| | | *Sugar B.** | | | |
| 25 c. c. | 0·1413 gram. | 0·0719 gram. | ----- | 0·2876 gram. | 2·87 |
| 25 | 0·1400 | 0·0713 | ----- | 0·2852 | 2·85 |
| | | *Total carbohydrates A.* | | | |
| 25 c. c. | 0·1560 gram. | 0·0796 gram. | ----- | 0·6368 gram. | 6·36 |
| 25 | 0·1585 | 0·0809 | ----- | 0·6472 | 6·47 |
| | | *Total carbohydrates B.* | | | |
| 25 c. c. | 0·1445 gram. | 0·0736 gram. | ----- | 0·5888 gram. | 5·88 |
| 25 | 0·1427 | 0·0726 | ----- | 0·5808 | 5·80 |

Following are the average percentage results:

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 25 grams. | With peptones and blood (A), | 1·65 % | ---- | 6·42 % |
| 25 | Without peptones and blood (B), | 1·54 | 2·86 % | 5·84 |
| | | +0·11 | | +0·58 |

In this experiment there is the same slight increase of total carbohydrates in the presence of peptone noticed in the two preceding experiments. The increase, however, is not great, and it suggests at once the question, whether the differences, although constant, are

* Sugar *A* was lost.

beyond the ordinary limits of error. This question we have endeavored to answer in the next experiment.

### *Experiment* IV.

A rabbit's liver removed from the body immediately after death, was prepared in the usual manner. Two mixtures, exactly alike, were then made as follows:

| *A.* | *B.* |
|---|---|
| 25 grams of liver. | 25 grams of liver. |
| 100 c. c. of water. | 100 c. c. of water. |

These were in the bath 23 minutes after the death of the animal and were warmed at 40° C. for 2 hours. The two portions were then extracted and analyzed as in the preceding experiments; the object being to see how great a variation would be obtained by this like treatment of the two portions of sampled liver. Following are the results:

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·1575 gram. | 0·0802 gram. | 0·0721 gram. | 0·5768 gram. | 5·76 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·1585 gram. | 0·0809 gram. | 0·0728 gram. | 0·5824 gram. | 5·82 |
| | | *Sugar A.* | | | |
| 10 c. c. | 0·0433 gram. | 0·0225 gram. | ----- | 0·2250 gram. | 2·25 |
| | | *Sugar B.* | | | |
| 10 c. c. | 0·0430 gram. | 0·0224 gram. | ----- | 0·2240 gram. | 2·24 |
| | | *Total carbohydrates A.* | | | |
| 25 c. c. | 0·2340 gram. | 0·1207 gram. | ----- | 0·9656 gram. | 9·65 |
| | | *Total carbohydrates B.* | | | |
| 25 c. c. | 0·2335 gram. | 0·1203 gram. | ----- | 0·9624 gram. | 9·62 |

*Percentage results.*

| | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|
| *A.* | 5·76 per cent. | 2·25 per cent. | 9·65 per cent. |
| *B.* | 5·82 | 2·24 | 9·62 |
| | −0·06 | +0·01 | +0·03 |

These results plainly show that when the conditions of the experiment are exactly the same, the average variation in results will be considerably less than 0·1 per cent. Consequently variations greater than this must have their origin in something other than the ordinary errors of analysis. Hence, in the three preceding experiments we have to account for an average increase of about 0·5 per cent. in

total carbohydrates in those cases where peptone and blood are both present.

A comparison of Seegen's results* show that while an aqueous solution of peptone alone in contact with fresh liver increases somewhat the percentage of both sugar and total carbohydrates, the addition of blood, kept arterial by the passage of a current of air through the fluid, appears to still further increase the percentage of sugar and carbohydrates. Seegen, moreover, shows by a blank experiment, that blood alone in contact with the liver has no more influence on the formation of carbohydrates than distilled water.

### *Experiment* V.

This experiment was tried mainly to see what influence peptones by themselves in the absence of blood, would have on the formation of sugar and total carbohydrates. Two portions of sampled liver from a large rabbit were treated as follows:

| *A.* | *B.* |
|---|---|
| 50 grams liver. | 50 grams liver. |
| 50 c. c. of water containing 2 grams of peptones. | 50 c. c. of water. |

The solution of peptone was poured over the liver just 45 minutes after the death of the animal. The mixtures were placed in a bath at 40° C. for 3 hours, after which they were allowed to stand at the temperature of the room for 21 hours. They were then extracted and analyzed in the usual manner, with the following results:

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·1900 gram. | 0·0973 gram. | 0·0875 gram. | 0·7000 gram. | 3·50 |
| 25 | 0·1915 | 0·0980 | 0·0882 | 0·7056 | 3·52 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·1785 gram. | 0·0913 gram. | 0·0821 gram. | 0·6568 gram. | 3·28 |
| | | *Sugar A.* | | | |
| 25 c. c. | 0·4085 gram. | 0·2177 gram. | ----- | 0·8708 gram. | 4·35 |
| 10 | 0·1735 | 0·0887 | ----- | 0·8870 | 4·43 |
| | | *Sugar B.* | | | |
| 10 c. c. | 0·1745 gram. | 0·0892 gram. | ----- | 0·8920 gram. | 4·46 |
| | | *Total carbohydrates A.* | | | |
| 10 c. c. | 0·1985 gram. | 0·1017 gram. | ----- | 2·0340 grams. | 10·17 |
| | | *Total carbohydrates B.* | | | |
| 10 c. c. | 0·1830 gram. | 0·0937 gram. | ----- | 1·8740 grams. | 9·37 |

* Pflüger's Archiv, vol. xxv, p. 172; ibid, vol. xxviii, p. 125.

*Average percentage results.*

| Amount of liver taken | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams | With peptones (A), | 3·51 per cent. | 4·39 per cent. | 10·17 per cent. |
| 50 | Without peptones (B), | 3·28 | 4·46 | 9·37 |
| | | +0·23 | −0·07 | +0·80 |

## *Experiment* VI.

This experiment is practically a repetition of the preceding one, excepting that the temperature throughout the experiment was about 18–20° C. and the length of time 24 hours. This latter point we deem of considerable importance, for as the results show, the same increase in total carbohydrates in the presence of peptone is apparent here, after 24 hours treatment and also in the preceding experiment after 21 hours treatment, as has been observed at the end of 2 to 3 hours in the presence of peptone and blood. For Seegen lays considerable stress upon the fact that in many animals the newly-formed carbohydrates and sugar, supposed to have their origin in the peptones, are after a time decomposed, so that at the end, say of 24 hours, the content of sugar and total carbohydrates fall back to their original amount, that is, the amount found in the control. Seegen further claims that this point speaks strongly in favor of the action of the liver, as such, on the conversion of peptone, and rabbits' liver according to his experiments is no exception to the rule.* The experiment was as follows:

| *A.* | *B.* |
|---|---|
| 40 grams fresh liver (rabbit). | 40 grams fresh liver. |
| 55 c. c. of water containing 2 grams of peptone. | 55 c. c. of water. |

These two mixtures stood at the temperature of the room for 24 hours, when they were extracted and analyzed with the following results:

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·2767 gram. | 0·1436 gram. | 0·1292 gram. | 1·0336 grams. | 6·46 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·2515 gram. | 0·1299 gram. | 0·1168 gram. | 0·9344 gram. | 5·84 |
| | | *Sugar A.* | | | |
| 25 c. c. | 0·2745 gram. | 0·1424 gram. | ----- | 0·5696 gram. | †3·56 |
| 25 | 0·2655 | 0·1375 | ----- | 0·5500 | 3·43 |
| | | *Sugar B.* | | | |
| 25 c. c. | 0·3210 gram. | 0·1681 gram. | -- -- | 0·6724 gram. | 4·20 |
| 25 | 0·3260 | 0·1709 | ----- | 0·6836 | 4·27 |
| | | *Total carbohydrates A.* | | | |
| 10 c. c. | 0·2100 gram. | 0·1079 gram. | ----- | 2·1580 grams. | 13·48 |
| | | *Total carbohydrates B.* | | | |
| 10 c. c. | 0·2013 gram. | 0·1032 gram. | ----- | 2·0640 grams. | 12·90 |

* Pflüger's Archiv, vol. xxv, p. 175.

† In this determination, difficulty was experienced in obtaining a good reduction.

*Average percentage results.*

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams | With peptones (A), | 6·46 per cent. | 3·49 per cent. | 13·48 per cent. |
| 40 | Without peptones (B), | 5·84 | 4·23 | 12·90 |
| | | +0·62 | −0·74 | +0·58 |

Both of these experiments tend to show that the presence of blood has no especial influence on the percentage of total carbohydrates; fully as great an increase is to be noticed in the presence of peptone without blood, as when the latter is present. Evidently then, if the increase in total carbohydrates noticed in all of our experiments is really due to the post-mortem formation of carbohydrate matter from peptone it is quite certain that blood is not at all essential to the reaction, at least in the livers of rabbits.

It is to be noticed, moreover, in the two last experiments that, in the absence of blood, the sugar is not increased in amount in the presence of peptone. On the contrary, the presence of peptone under such conditions appears to diminish the formation of sugar, glycogen being correspondingly increased. In all of the experiments with rabbits, it is apparent from the results, that any increase of sugar in the presence of peptone is in every instance counterbalanced by a corresponding decrease in glycogen. In the last two experiments, the same relationship between the amount of glycogen and sugar is to be noticed, only here the greatest percentage of sugar is to be found in that portion of the liver which was treated without peptones. This would suggest that blood either facilitates in some manner the action of such amylolytic ferment as is present in the liver, or else that it introduces an additional ferment which causes increased amylolytic action. Blood certainly does not contain any substance convertible into sugar by the action of boiling acids, since the increase in total carbohydrates is no greater in the presence of blood than in the presence of peptone alone.

We have therefore tried the following experiment in order to ascertain whether blood by itself, in the absence of peptone, has any influence whatever on the formation of sugar.

### *Experiment* VII.

| *A.* | *B.* |
|---|---|
| 50 grams of sampled liver (rabbit). | 50 grams of sampled liver. |
| 27 grams of blood. | 92 c. c. of water. |
| 65 c. c. of water. | |

The blood from the same rabbit was poured over the liver 35 minutes after the death of the animal and the two flasks containing the

mixtures were then placed at a temperature of 40° C. for 2 hours. A constant current of air was kept passing through the blood during this time. Following are the results obtained:

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·1270 gram. | 0·0647 gram. | 0·0582 gram. | 0·4656 gram. | 2·32 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·1947 gram. | 0·0997 gram. | 0·0897 gram. | 0·7176 gram. | 3·58 |
| | | *Sugar A.* | | | |
| 10 c. c. | 0·1635 gram. | 0·0845 gram. | ----- | 0·8450 gram. | 4·22 |
| | | *Sugar B.* | | | |
| 10 c. c. | 0·1420 gram. | 0·0723 gram. | ----- | 0·7230 gram. | 3·61 |
| 10 | 0·1415 | 0·0720 | ----- | 0·7200 | 3·60 |
| | | *Total carbohydrates A.* | | | |
| 20 c. c. | 0·3800 gram. | 0·2014 gram. | ----- | 2·0140 | 10·07 |
| | | *Total carbohydrates B.* | | | |
| 20 c. c. | 0·3785 gram. | 0·2005 gram. | ----- | 2·0050 | 10·02 |

*Average percentage results.*

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With blood (A), | 2·32 per cent. | 4·21 per cent. | 10·07 per cent |
| 50 | Without blood (B), | 3·58 | 3·61 | 10·02 |
| | | −1·26 | +0·60 | +0·05 |

Total carbohydrates are not at all affected by the presence of blood, but the percentage of sugar is considerably increased, and in accord with the increase of sugar, is to be noticed a decided decrease in the percentage of glycogen. Evidently then the percentage of sugar in the rabbit's liver is increased in the presence of blood, which increase is due wholly to a more vigorous decomposition of glycogen. Moreover, whenever increase of sugar has been observed in our experiments a corresponding decrease in glycogen has as a rule, also been seen. In this respect, therefore, our results agree with those of Boehm and Hoffmann, as also with those of Delprat. Seegen, however, states in a later communication,* that in rabbits, glycogen is more rapidly changed than in the case of dogs, or in other words that the liver-glycogen of rabbits is less resistant to the action of ferments.

In all of the preceding experiments with the livers of rabbits, it is to be noticed that the sum of glycogen, calculated as dextrose, and

* Pflüger's Archiv, vol. xxiv, p. 467.

the sugar, is not at all equal to the figures representing total carbohydrates, being in every instance considerably less than the latter. Thus in Experiment V, the amount of total carbohydrates is in *A* (with peptones) 10·17 per cent., while the sum of sugar and glycogen calculated as dextrose is but 8·29 per cent.; a deficit of 1·88 per cent. In *B*, likewise, where peptones are not present, there is a similar deficit, amounting in this case to 1·27 per cent. It is not to be supposed that such a deficiency could in any manner arise from errors of analysis and the most natural supposition is that the sugar, determined and calculated as dextrose, might be of lower reducing power; or in other words that it might consist of maltose instead of dextrose, or rather, of a mixture of maltose and dextrose or of a soluble dextrin. O. Nasse* has stated that the dead liver contains dextrose, or a sugar whose reducing power is not increased by heating with dilute sulphuric acid. Seegen and Kratschmer† also state that the dead liver contains dextrose, and further, that the liver sugar is *exclusively* dextrose. This opinion is based mainly upon the fact that the fluid obtained from a calf's liver by pressure, yielded by dialysis and subsequent treatment with alcohol, a saccharine body, which on the addition of an alcoholic solution of potash was converted into the known *dextrose-potash* compound. Musculus and V. Mering,‡ however, claim that in addition to dextrose, the dead liver also contains maltose. This sugar they detected twice; once in the dead liver of a dog, 1 hour after death, and again, also in a dog's liver, 5 hours after death. In both cases dextrose was likewise present. Dextrin they were not able to detect with certainty, but they consider that the liver ferment also forms this body, intermediate between glycogen and the sugars. E. Külz§ has also prepared from the dead livers of dogs pure dextrose, but he does not conclude definitely as to the presence of dextrin and maltose. It is to be seen, therefore, that while there is unanimity of opinion regarding the presence of dextrose, there is less certainty regarding the presence of the lower reducing sugar, maltose.

We have therefore, carefully examined the nature of the sugar remaining in the alcoholic filtrate after the precipitation of glycogen, and we find, in almost every instance, that the saccharine body there

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* Bemerkungen zur Physiologie der Kohlehydrate. Pflüger's Archiv, vol. xiv, p. 473

† Die Natur des Leberzucker. Pflüger's Archiv, vol. xxii, p. 214.

‡ Ueber die Umwandlung von Stärke und Glycogen durch Diastas, Speichel, Pancreas und Leberferment. Zeitschrift für physiologische Chemie, vol. ii, p. 417.

§ Ueber die Natur des Zuckers in der todtenstarren Leber. Pflüger's Archiv, vol. xxiv, p. 52.

present, has its reducing power considerably increased by heating for a short time with 2 per cent. sulphuric acid. Moreover, the sum of glycogen calculated as dextrose, and the liver sugar converted wholly into dextrose by boiling with dilute acid, exactly equals the total carbohydrates in those cases where peptones are not present. Thus in Experiment V, *A* and *B*, the final sugar solution amounted in each instance to 100 c. c.; 50 c. c. of these solutions were mixed with sufficient 10 per cent. sulphuric acid to insure a content of 2 per cent., after which the two solutions were boiled for two hours, evaporation being prevented by an inverted Liebig's condenser. On cooling, the solutions were made nearly neutral, concentrated somewhat and finally brought back to a volume of exactly 50 c. c. Following are the analytical results both before and after boiling with the dilute acid.

| | Volume used. | Weight Cu. | Equivalent in dextrose. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *A. With peptones.* | | | |
| Before boiling, | 10 c. c. | 0·1735 gram. | 0·0887 gram. | 0·8870 gram. | 4·43 |
| After boiling, | 10 | 0·2270 | 0·1169 | 1·1690 | 5·84 |
| | | *B. Without peptones.* | | | |
| Before boiling. | 10 c. c. | 0·1745 gram. | 0·0892 gram. | 0·8920 gram. | 4·46 |
| After boiling, | 10 | 0·2234 | 0·1150 | 1·1500 | 5·75 |

Hence, it is evident that the liver sugar, in this instance at least, is not made up entirely of dextrose. Neither is it wholly maltose, for if such were the case, the reducing power before and after boiling with acid, would be in the proportion of 66 : 100, whereas in the above experiment the ratio in *A* is 76·4 : 100 and in *B* 78·1 : 100. Still, it would appear that the lower reducing body is present in the largest quantity.

It was not our purpose to study particularly the nature of the liver sugar, so we have not sought for positive proof of the character of this lower reducing body. That it is not dextrin, or glycogen left unprecipitated by the alcohol, is evident from the fact that the addition of a little pure yeast to the normal sugar solution sets up a fermentation by which the sugars are not only completely decomposed but no amylaceous bodies whatever remain in the fluid; at least none which will yield reducing bodies on boiling with dilute sulphuric acid. Consequently it would appear that the lower reducing body is in all probability maltose. We do not intend to say, however, that dextrin is never present in the liver; on the contrary, we are inclined to agree with Musculus and v. Mering that dextrin is doubtless formed as an antecedent to maltose, but in the two or

three fermentation experiments that we have tried, the sugar solution has never contained dextrin. It would of course be more natural in seeking for dextrin to look in the glycogen precipitate. Finally, it is interesting to notice in connection with this same experiment, that in *B* the sum of glycogen calculated as dextrose and the sugar after boiling with sulphuric acid equals the total carbohydrates found; while in *A* where peptones are present, the total carbohydrates more than equal the sum of glycogen and sugar, as is shown by the following figures from Experiment V.

| | Sugar not boiled with acid. | | Sugar boiled with acid. | |
|---|---|---|---|---|
| | *A.* | *B.* | *A.* | *B.* |
| Glycogen calculated as dextrose, | 3·90 p. c. | 3·64 p. c. | 3·90 p. c. | 3·64 p. c. |
| Sugar calculated as dextrose, | 4·43 | 4·46 | 5·84 | 5·75 |
| | 8·33 | 8·10 | 9·74 | 9·39 |

Total carbohydrates found in *A* 10·17 per cent.
" " " *B* 9·37

This shows an apparent increase in total carbohydrates of 0·43 per cent. (in A) under the influence of the peptones; this being the difference between the total carbohydrates found, and the sum of glycogen calculated as dextrose and the sugar after boiling with dilute acid. In *B* on the other hand, the total carbohydrates found and the sum of glycogen and sugar differ but 0·02 per cent.

## *Experiment* VIII.

This experiment was tried mainly to verify the preceding statements regarding the liver sugar.

40 grams of liver from a rabbit were finely divided and placed in a flask with 50 c. c. of water. The mixture was then warmed at 38–40° C. for 2 hours, when it was boiled and extracted in the usual manner. The liver was placed in the warm bath just 25 minutes after the death of the rabbit, consequently 2½ hours intervened between the death of the animal and the stopping of ferment action. Following are the results of the analysis:

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen.* | | | |
| 25 c. c. | 0·2990 gram. | 0·1560 gram. | 0·1404 gram. | 1·1232 grams. | 7·02 |
| | | *Sugar.* | | | |
| 10 c. c. | 0·0915 gram. | 0·0466 gram. | ----- | 0·4660 gram. | 2·91 |
| | | *Sugar after boiling with dilute* $H_2SO_4$. | | | |
| 10 c. c. | 0·1145 gram. | 0·0582 gram. | ----- | 0·5820 gram. | 3·63 |
| | | *Total carbohydrates.* | | | |
| 10 c. c. | 0·1790 gram. | 0·0916 gram. | ----- | 1·8320 grams. | 11·45 |

| | | | |
|---|---|---|---|
| Glycogen as dextrose, | 7·80 per cent. | | 7·80 per cent. |
| Sugar, | 2·91 | After boiling with $H_2SO_4$. | 3·63 |
| | 10·71 | | 11·43 |

It is evident that here, as in the preceding experiment, the sum of glycogen calculated as dextrose and the sugar equals the total carbohydrates actually found, only when the sugar has been boiled with dilute sulphuric acid, in which case the agreement is, as before, almost exact. Plainly then, dextrose is not the only sugar formed in the liver, and if, as seems probable from our experiments, the sugar has its origin in the hepatic glycogen, it would be quite in accord with analogy to expect the presence of both maltose and dextrose.

The relative reducing power, before and after boiling with acid, is much the same as in the preceding experiments, viz: 79·9 : 100, and indicates the presence of considerable of the body with lower reducing power. This portion of the experiment was duplicated with another 40 grams of liver from the same rabbit under exactly the same conditions as to time and temperature, with results almost identical with those obtained from the preceding portion, thus testifying to the accuracy of the methods.

*Sugar solution before boiling with dilute acid.*

10 c. c. gave 0·0865 gram Cu=0·0444 gram dextrose=2·78 per cent.

*Sugar solution after boiling with dilute acid.*

10 c. c. gave 0·1080 gram Cu=0·0550 gram dextrose=3·44 per cent.

The relative reducing power before and after boiling with dilute sulphuric acid is in this case 80·0 : 100.

Experiments were now tried, to ascertain the influence of peptone on the formation of carbohydrates in the livers of other animals.

## *Experiment* IX.

A large cat in full digestion, fed mainly on proteid matter, was chloroformed, blood collected from the jugular vein and the liver at once removed. Two mixtures were then prepared as follows:

| *A.* | *B.* |
|---|---|
| 50 grams sampled liver. | 50 grams sampled liver. |
| 20 grams blood, | 100 c. c. of water. |
| 50 c. c. of a 10 per cent. solution of peptone. | |
| 40 c. c. of water. | |

These were placed in a bath at 38–40° C. 50 minutes after the death of the animal, and were kept there for 2½ hours, with a constant current of air passing through *A* in order to render the blood

arterial. At the end of this time further action was prevented by boiling the mixtures, after which they were extracted in the usual manner. Neither of the two extracts contained enough glycogen for estimation. Following are the other results :

| Volume used. | Weight of Cu. | Equivalent in dextrose. | Total amount. | Per cent. |
|---|---|---|---|---|
| | | *Sugar A.* | | |
| 25 c. c. | 0·1710 gram. | 0·0874 gram. | 0·3496 gram. | 1·74 |
| | | *After boiling with dilute* $H_2SO_4$. | | |
| 25 c. c. | 0·1730 gram. | 0·0885 gram. | 0·3540 gram. | 1·77 |
| | | *Sugar B.* | | |
| 25 c. c. | 0·1638 gram. | 0·0837 gram. | 0·3348 gram. | 1·67 |
| | | *After boiling with dilute* $H_2SO_4$. | | |
| 25 c. c. | 0·1750 gram. | 0·0895 gram. | 0·3580 gram. | 1·79 |
| | | *Total carbohydrates A.* | | |
| 25 c. c. | 0·1048 gram. | 0·0533 gram. | 0·4264 gram. | 2·13 |
| | | *Total carbohydrates B.* | | |
| 25 c. c. | 0·0930 gram. | 0·0474 gram. | 0·3792 gram. | 1·89 |

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. Before boiling. | After boiling. | Total carbohydrates. |
|---|---|---|---|---|---|
| 50 grams. | With peptones and blood (A), | 0 | 1·74 % | 1·77 % | 2·13 % |
| 50 | Without peptones and blood (B), | 0 | 1·67 | 1·79 | 1·89 |
| | | | +0·07 | −0 02 | +0·24 |

In the presence of peptone, there is to be noticed a slight increase in total carbohydrates, which increase receives confirmation from the fact that in *A*, the total carbohydrates exceed the sugar by 0·36 per cent., while in *B* the difference after boiling the sugar with dilute acid is but 0·1 per cent., probably within the limits of error. It is to be noticed, moreover, that in the presence of peptone, the sugar formed is wholly dextrose, its reducing power not being materially changed by boiling with dilute acid, whereas in *B* there is a slight increase in reducing power on boiling with acid. If the difference is sufficient to warrant any explanation it might be suggested that peptone by its presence* stimulates the ferment which presumably converts maltose into dextrose and thus in *A* complete conversion was effected sooner than in *B*.

## *Experiment* X.

This experiment was like the preceding, with the exception that the cat employed was not in full digestion. The stomach was nearly

* Compare Chittenden and Smith, Trans. Conn Acad., vol. vi, p. 343.

empty and the small intestines likewise. The sampled liver was in the bath at 40° C., 25 minutes after the death of the animal.

| *A.* | *B.* |
|---|---|
| 40 grams of liver. | 40 grams of liver. |
| 25 grams of blood. | 135 c. c. of water. |
| 100 c. c. of a 2 per cent. solution of peptone. | |
| 10 c. c. of water. | |

These mixtures were warmed at 40° C. for 2 hours, with a current of air passing through *A*, after which they were extracted and treated in the usual manner. Glycogen was not found in sufficient quantity for determination in either *A* or *B*. An accident happened to the sugar solutions, so that the absolute amount of sugar could not be estimated, but the relative reducing power of the sugar solution, before and after boiling with dilute sulphuric acid, was accurately determined.

| Volume used. | Weight of Cu. | Equivalent in dextrose. | Total amount. | Per cent. |
|---|---|---|---|---|
| | | *Total carbohydrates A.* | | |
| 25 c. c. | 0·1070 gram. | 0·0545 gram. | 0·4360 gram. | 2·72 |
| | | *Total carbohydrates B.* | | |
| 25 c. c. | 0·0865 gram. | 0·0441 gram. | 0·3528 gram. | 2·20 |
| | | *Sugar A.* | | |
| 25 c. c. | 0·1045 gram. | 0·0529 gram. | Before boiling with dilute acid. | |
| 25 | 0·1479 | 0·0753 | After boiling with acid. | |
| | | *Sugar B.* | | |
| 25 c. c. | 0·1453 gram. | 0·0740 gram. | Before boiling with dilute acid. | |
| 25 | 0·1925 | 0·0986 | After boiling with acid. | |

In this case, the total carbohydrates show an increase of 0·52 per cent. in the presence of peptone. The sugars, unlike the result in the preceding experiment, show a very great increase in reducing power, on being boiled with dilute sulphuric acid. In *A*, the relative reducing power before and after boiling with the acid, is 70·6 : 100 and in *B* 75·4 : 100. Hence in this experiment it would appear that the lower reducing body, presumably maltose, is present in great excess.

### *Experiment* XI.

In this experiment, a liver was taken from a freshly killed lamb, sampled in the usual manner and then warmed at 40° C., with and without peptone, for 4 hours. The liver was in the bath and mixed with the peptone solution just one hour and 20 minutes after the death of the animal. In this experiment no blood was used.

| *A.* | *B.* |
|---|---|
| 50 grams of liver. | 50 grams of liver. |
| 50 c. c. of a 4 per cent. solution of peptone. | 50 c. c. of water. |

Glycogen could not be detected in sufficient quantity for estimation. Following are the other results:

| Volume used. | Weight of Cu. | Equivalent in dextrose. | Total amount. | Per cent. |
|---|---|---|---|---|
| | | *Sugar A.* | | |
| 25 c. c. | 0·2440 gram. | 0·1260 gram. | 0·5040 gram. | 2·52 |
| | | *After boiling with dilute* $H_2SO_4$. | | |
| 25 c. c. | 0·2495 gram. | 0·1289 gram. | 0·5156 gram. | 2·57 |
| | | *Sugar B.* | | |
| 25 c. c. | 0·2430 gram. | 0·1255 gram. | 0·5020 gram. | 2·51 |
| | | *After boiling with dilute* $H_2SO_4$. | | |
| 25 c. c. | 0·2445 gram. | 0·1262 gram. | 0·5048 gram. | 2·52 |
| | | *Total carbohydrates A.* | | |
| 25 c. c. | 0·1185 gram. | 0·0603 gram. | 0·4824 gram. | 2·41 |
| | | *Total carbohydrates B.* | | |
| 25 c. c. | 0·1210 gram. | 0·0616 gram. | 0·4928 gram. | 2·46 |

Here there is neither increase in total carbohydrates in the presence of peptone, nor is there any change in the reducing power of the sugar solutions after boiling with dilute acid. Total carbohydrates, moreover, fall a trifle below the percentage of sugar, although the difference is within the limits of error.

## *Experiment* XII.

With a sheep's liver, 24 hours after death.

| *A.* | *B.* |
|---|---|
| 50 grams of sampled liver. | 50 grams of sampled liver. |
| 100 c. c. of a 2 per cent. solution of peptone. | 100 c. c. of water. |

These were warmed at 40° C. for 1½ hours, then extracted and tested. No glycogen was found.

| Volume used. | Weight of Cu. | Equivalent in dextrose. | Total amount. | Per cent. |
|---|---|---|---|---|
| | | *Sugar A.* | | |
| 25 c. c. | 0·1085 gram. | 0·0552 gram. | 0·2208 gram. | 1·10 |
| | | *Sugar B.* | | |
| 25 c. c. | 0·0825 gram. | 0·0420 gram. | 0·1680 gram. | 0·84 |
| | | *Total carbohydrates A.* | | |
| 25 c. c. | 0·0750 gram. | 0·0383 gram. | 0·3064 gram. | 1·53 |
| | | *Total carbohydrates B.* | | |
| 25 c. c. | 0·0465 gram. | 0·0241 gram. | 0·1928 gram. | 0·96 |

| Amount of liver taken. | Method of treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones (A). | 0 | 1·10 per cent. | 1·53 per cent. |
| 50 | Without peptones (B). | 0 | 0·84 | 0·96 |
| | | | +0·26 | +0·57 |

Increase in both sugar and total carbohydrates is to be noticed in *A*. The increase in total carbohydrates, moreover, is twice as great as the increase in sugar.

### *Experiment* XIII.

With a calf's liver, from a freshly killed animal, obtained at the slaughter house.

| *A.* | *B.* | *C.* |
|---|---|---|
| 50 grams of liver. | 50 grams of liver. | 50 grams of liver. |
| 100 c. c. of water. | 100 c. c. of water. | 100 c. c. of a 2 per cent. solution of peptone. |

*A* was warmed at 40° C. for 1 hour and 20 minutes, after which it was extracted and analyzed; *B* and *C* on the other hand, were heated for 1 hour and 30 minutes at the same temperature, after which they were allowed to stand for 16 hours at about 18–20° C. before being extracted. The special object in view was to ascertain the influence of time on the disappearance of glycogen.

Following are the results obtained :

| Volume used. | Weight of Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| | | *Glycogen A.* | | | |
| 25 c. c. | 0·0230 gram. | 0·0125 gram. | 0·0112 gram. | 0·0896 gram. | 0·44 |
| | | *Glycogen B.* | | | |
| 25 c. c. | 0·0105 gram. | 0·0063 gram. | 0·0056 gram. | 0·0448 gram. | 0·22 |
| | | *Glycogen C.* | | | |
| 25 c. c. | a mere trace only. | | | | |
| | | *Sugar A.* | | | |
| 25 c. c. | 0·1900 gram. | 0·0973 gram. | ----- | 0·3892 gram. | 1·94 |
| | | *After boiling with dilute* $H_2SO_4$. | | | |
| 25 c. c. | 0·2100 gram. | 0·1079 gram. | ----- | 0·4316 gram. | 2·15 |
| | | *Sugar B.* | | | |
| 25 c. c. | 0·2445 gram. | 0·1262 gram. | ----- | 0·5048 gram. | 2·52 |
| | | *After boiling with dilute* $H_2SO_4$. | | | |
| 25 c. c. | 0·2535 gram. | 0·1310 gram. | ----- | 0·5240 gram. | 2·62 |
| | | *Sugar C.* | | | |
| 25 c. c. | 0·2315 gram. | 0·1198 gram. | ----- | 0·4792 gram. | 2·39 |
| | | *After boiling with dilute* $H_2SO_4$. | | | |
| 25 c. c. | 0·2620 gram. | 0·1357 gram. | ----- | 0·5428 gram. | 2·71 |
| | | *Total carbohydrates A.* | | | |
| 25 c. c. | 0·1210 gram. | 0·0616 gram. | ----- | 0·4928 gram. | 2·46 |
| | | *Total carbohydrates B.* | | | |
| 25 c. c. | 0·1410 gram. | 0·0718 gram. | ----- | 0·5744 gram. | 2·87 |
| | | *Total carbohydrates C.* | | | |
| 25 c. c. | 0·1405 gram. | 0·0715 gram. | ----- | 0·5720 gram. | 2·86 |

| | *A.* Without peptones, 1 hour 20 min. at 40° C. | *B.* Without peptones, 1½ hours at 40° C. and 16 h. at 20° C. | *C.* With peptones, 1½ hours at 40° C. and 16 h. at 20° C. |
|---|---|---|---|
| Glycogen | 0·44 per cent. | 0·22 per cent. | 0 |
| Sugar before boiling with $H_2SO_4$ | 1·94 | 2·52 | 2·39 per cent. |
| Sugar after boiling with $H_2SO_4$ | 2·15 | 2·62 | 2·71 |
| Total carbohydrates | 2·46 | 2·87 | 2·86 |

A comparison of these results shows no increase whatever in total carbohydrates in the presence of peptone (*B* and *C*.) Somewhat strange is the apparent increase of 0·41 per cent., in the absence of peptone after standing 24 hours. The percentage of glycogen is diminished one-half after standing 24 hours, and, as has been noticed several times before, it is still further diminished in the presence of peptone. Consistent with the theory of the formation of liver sugar from the hepatic glycogen, is the fact that in this experiment increase in sugar accompanies decrease in glycogen. This is shown best in the percentages of sugar, after boiling with dilute sulphuric acid. The relative reducing power of the sugar solutions, before and after boiling with dilute acid, is in *A* 90·1 : 100, in *B* 96·4 : 100, and in *C* 88·3 : 100. In other words the liver sugar in *B* after standing 24 hours is composed mainly of dextrose, the lower reducing body present in larger quantity in *A* having been gradually changed by longer contact with the liver tissue. In *C*, however, although the time was the same as in *B*, the presence of peptone, while it appears to increase the decomposition of glycogen and thus the actual amount of sugar formed, tends to prevent apparently the conversion of the lower reducing sugar into dextrose; hence in *C* the percentage of sugar before boiling with acid is less than in *B*, while after treatment with acid it is greater.

*Following is a resumé of the various results obtained with peptones.*

*Experiment* I.—Rabbit's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams. | With peptones and blood. | 5·46 % | 2·91 % | 11·08 % |
| 40 | Without " " | 6·21 | 2·74 | 10·75 |
| | 2 hours at 40° C. | −0·75 | +0·17 | +0·33 |

*Experiment* II.—Rabbit's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates |
|---|---|---|---|---|
| 40 grams. | With peptones and blood. | 7·46 % | 3·26 % | 14·15 % |
| 40 | Without " " | 8·09 | 2·75 | 13·55 |
| | 2 hours at 40° C. | −0·63 | +0·51 | +0·60 |

*Experiment* III.—Rabbit's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 25 grams. | With peptones and blood. | 1·65 % | --- | 6·42 % |
| 25 | Without " " | 1·54 | 2·86 % | 5·84 |
| | 2 hours at 40° C. | +0·11 | | +0·58 |

*Experiment* V.—Rabbit's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones. | 8·51 % | 4·39 % | 10·17 % |
| 50 | Without " | 8·28 | 4·46 | 9·37 |
| | 3 hours at 40° C. | +0·23 | −0·07 | +0·80 |

After boiling the sugar with dilute $H_2SO_4$, 4·39=5·82 % Ratio 76·4:100.
" " " " 4·46=5·75 " 78·1:100.
+0·07

*Experiment* VI.—Rabbit's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams. | With peptones. | 6·46 % | 3·49 % | 13·48 % |
| 40 | Without " | 5·84 | 4·23 | 12·90 |
| | 24 hours at 18–20° C. | +0·62 | −0·74 | +0·58 |

*Experiment* IX.—Cat's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones and blood. | 0 | 1·74 % | 2·13 % |
| 50 | Without " " | 0 | 1·67 | 1·89 |
| | 2½ hours at 40° C. | | +0·07 | +0·24 |

After boiling the sugar with dilute $H_2SO_4$, 1·74=1·77 % Ratio 98·8:100.
" " " " " 1·67=1·79 " 93·6:100.
−0·02

*Experiment* X.—Cat's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 40 grams. | With peptones and blood. | 0 | ---- | 2·72 % |
| 40 | Without " " | 0 | ---- | 2·20 |
| | 2 hours at 40° C. | | | +0·52 |

Ratio before and after boiling with dilute $H_2SO_4$, 70·6:100, with peptones.
" " " " 75·4:100, without peptones.

*Experiment* XI.—Lamb's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones. | 0 | 2·52 % | 2·41 % |
| 50 | Without " | 0 | 2·51 | 2·46 |
| | 4 hours at 40° C. | | +0·01 | −0·05 |

After boiling the sugar with dilute $H_2SO_4$, 2·52=2·57%
" " " " 2·51=2·52

*Experiment* XII.—Sheep's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones. | 0 | 1·10 % | 1·53 % |
| 50 | Without " | 0 | 0·84 | 0·96 |
| | 1½ hours at 40° C. | | +0·26 | +0·57 |

*Experiment* XIII.—Calf's liver.

| Liver taken. | Treatment. | Glycogen. | Sugar. | Total carbohydrates. |
|---|---|---|---|---|
| 50 grams. | With peptones. | 0 | 2·89 % | 2·86 % |
| 50 | Without " | 0·22 % | 2·52 | 2·87 |
| | 1½ hours at 40° C. and | —— | —— | —— |
| | 16 hours at 20° C. | −0·22 | −0·18 | −0·01 |

After boiling the sugar with dilute $H_2SO_4$, 2·89=2·71 % Ratio 88·8:100.
" " " " 2·52=2·62 " 96·4:100.
+0·09

These results show throughout no indications whatever of a formation of sugar from peptones; on the contrary the results are wholly in accord with the formation of liver sugar from the hepatic glycogen. At the same time it is apparent in several cases, that the decrease of glycogen is somewhat in excess of the increase in sugar, which fact would agree with the view of Seegen and Kratschmer that the destroyed glycogen is consumed in some other manner than by conversion into sugar. But this is in the liver after death, from which we cannot assume like action during life. There is further, in the results obtained, strong evidence that the liver sugar, as found after death, is a mixture of maltose and dextrose; the former presumably being converted into the latter by further ferment action. As to the total carbohydrates the results certainly accord in a general way with those obtained by Seegen. In nearly every case, the presence of peptone gives rise to an increase in total carbohydrates; the average increase in 8 experiments is, however, but 0·52 per cent. This increase, though small, is certainly too large to be explained by the assumption of analytical errors, and the increase, moreover, is too constant to admit of such an explanation. Futhermore, it is to be noticed in several instances, that in the presence of peptone the total carbohydrates found are greater than the sum of the boiled sugar and glycogen calculated as dextrose, while in the control they are practically equal; a fact which certainly lends favor to the view that the apparent increase of total carbohydrates is a real increase, due in some manner to the presence of peptone. Opposed to this view, however, or rather to the view that the liver possesses the power of

changing peptone into carbohydrates is the fact that in our experiments the same increase of carbohydrates is to be noticed after 24 hours' contact with peptone as after a shorter time; in fact in our experiments, time appears to have no noticeable influence on the total carbohydrates whatever, unless the like results in Experiment XIII are taken as confirmation of Seegen's statement that after a time the newly formed sugar is decomposed and thus the content of total carbohydrates falls back to its original amount; but in this experiment there is no evidence that the total carbohydrates ever were larger in amount. While our results therefore corroborate Seegen's and Kratschmer's statements regarding an increase of carbohydrates in the presence of peptone, we cannot consider that the slight increase in question, far less than that recorded in their experiments, is sufficiently pronounced to decide conclusively upon such an important theory. Furthermore, the noticeable lack of increase in sugar in the presence of peptone, excepting such increase as is attended with decrease in glycogen, is an additional reason for not attaching as much importance to the slight increase in total carbohydrates alone, as might otherwise be done. Consequently we must conclude that, in our opinion, the results obtained in these experiments do not warrant the adoption of this theory regarding the origin of the liver sugar, and that without further proof to the contrary we must still adhere to the formation of liver sugar from the hepatic glycogen.

## XIV.—Globulin and Globulose Bodies.* By W. Kühne, Professor of Physiology in the University of Heidelberg, and R. H. Chittenden.

The following investigation is a continuation of our previous work with the albumose bodies,† and constitutes the commencement of a study of the various primary cleavage products, formed by the action of pepsin from the better characterized and purer albumins. Our work in this direction was at first begun with the albuminous bodies occurring in blood serum, egg albumin, and in fibrin and from the results then obtained we were led to the conviction‡ that an extension of the work to the single albumins was necessary, particularly to serum albumin, globulin and myosin. In the meantime, our first conjecture that hemialbumose might possibly be a mixture, had been established and led finally to the isolation of the albumose bodies of fibrin.§ At this stage of our work, fibrin was the only albuminous material employed because it was the simplest to prepare, was easily digestible, and hitherto had been mostly used in digestion experiments. These practical considerations, to which must be added the fact that fibrin albumose was later found as a commercial article, were joined with the advantage of being able to make use of, and at the same time extend, the discoveries in the chemistry of digestion which have for decades been made with this particular albuminous material. Other advantages for the use of fibrin in this connection cannot well be claimed, since its peculiar position between the genuine and the coagulated albumins, its contamination with globulin and the presence of nuclein and other components of the white blood corpuscles tend to render it perhaps least fitted for use in an exact study of albumin cleavage; and since fibrin is of importance as a food stuff mainly in the form of the commercial so-called peptone, there are many reasons for proceeding to the study of other digestible substances for further results.

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* This and the following article were originally published in the Zeitschrift für Biologie, Band xxii. They are republished here to render the series complete in English.

† See Amer. Chem. Jour., vol. vi, p. 3.

‡ Zeitschrift für Biologie, Band xix, p. 159.

§ Ibid., xx, p. 11.

As we hope to show, however, the results gained in the study of the albumose bodies from fibrin are by no means to be considered as valueless, but on the other hand as quite advantageous in the treatment of the single albumins, particularly as an aid in the separation of the individual albumose bodies. Thus in our study of globulin, now to be described, we were able to use methods already tested.

The new method of precipitating albumose bodies by ammonium sulphate, which has meanwhile been discovered and which is certainly particularly advantageous, has not been used in our study of the globulose bodies, since these experiments were concluded before the introduction of that method. According to observations made by Dr. Neumeister in the Physiological Institute at Heidelberg, ammonium sulphate appears particularly well adapted to the purification of deuteroalbumose, since after complete removal of protoalbumose by sodium chloride and acid, deuteroalbumose alone is precipitated by addition of the ammonium salt. As, however, we had accomplished a separation of the different globulose bodies in another manner, it seemed unnecessary to use, in addition, the new method.

*Globulin.*

From the list of albuminous bodies which occur in sufficient quantity, and which at the same time admit of isolation without too great difficulty we have chosen for our first study, globulin. The substance was prepared from the serum of ox blood by the method of Hammarsten, in which repeated quantities of fresh serum were treated with an excess of crystallized magnesium sulphate at a temperature of 30° C. The precipitate so obtained was collected on a filter, washed with a saturated solution of the salt, pressed, dissolved in water, and reprecipitated a second time by the addition of magnesium sulphate. The precipitate was then dissolved in water, a little thymol added, and the solution dialyzed in running water in order to remove the magnesium salt. The final solution was then concentrated at 40° C., after which the globulin was precipitated with alcohol, washed with alcohol and finally extracted with ether. The substance so prepared, and which still contained some magnesium sulphate, was employed in the digestion experiments. A portion of the preparation, dried at 110° C. *in vacuo*, gave by analysis the results contained in the following table. The methods of analysis were the same as those employed in our previous work.*

* Compare Zeitschrift für Biologie, Band xx. p. 11, and Amer. Chem. Jour., vol vi, p. 3.

ANALYSIS OF GLOBULIN.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of ash. % of substance. | $BaSO_4$ after fusion with KOH + $KNO_3$ gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·6310 | 0·3833 | 6·74 | 1·1425 | 49·37 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·5122 | 0·3120 | 6·76 | 0·9270 | 49·35 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·8265 | ---- | ---- | ---- | ---- | 94·63 | 4·6 | 761·6 | 14·17 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·5773 | ---- | ---- | ---- | ---- | 64·85 | 5·5 | 773·8 | 14·08 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 1·4916 | ---- | ---- | ---- | ---- | ---- | -- | --- | ---- | 0·0520 | 3·49 | 0·1010 | 0·93 | ---- | ---- | ---- |
| VI | 1·1170 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | 0·0387 | 3·47 | 0·0756 | 0·92 | ---- | ---- | ---- |
| VII | 0·8445 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | 0·1543 | 2·50 | 1·57 |
| VIII | 0·5032 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0919 | 2·50 | 1·58 |

*Percentage composition of ash-free Globulin.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·15 | 51·13 | ---- | --- | ---- | ---- | *51·14* |
| H | 6·99 | 7·01 | ---- | ---- | ---- | ---- | *7·00* |
| N | ---- | ---- | 14·68 | 14·59 | ---- | ---- | *14·64* |
| S | ---- | ---- | ---- | ---- | 1·66 | 1·67 | *1·67* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *25·55* |
| | | | | | | | *100·00* |

*Digestion of Globulin.*

The preparation proved to be extremely difficult of digestion; this fact, however, enabled us to remove from the globulin the magnesium sulphate still remaining, since the large quantity of dilute hydrochloric acid added with the small amount of pepsin, failed at first to dissolve any of the albuminous matter. The undigested residue was then finally treated with an artificial gastric juice much richer in pepsin. 250 grams of the powdered globulin were at first warmed with 5 litres of 0·2 per cent. hydrochloric acid for 24 hours, whereby the substance swelled up to double its former bulk, but only a trace was dissolved; barely enough for a filtered portion to give with nitric acid and heat a slight turbidity. By the addition of 200 c. c. of normal gastric juice* to the swollen mass and warming it at 40° C. for 24 hours longer, the albuminous matter was not appreciably changed; a filtered portion, however, became turbid on neutralization and a small amount of albumose could be detected both with sodium chloride and with nitric acid. The entire mass of swollen globulin was then collected on a cloth filter, washed thoroughly with 0·2 per cent. hydrochloric acid and warmed again at 40° C. for six days with 4 litres of a particularly active gastric juice, containing 0·4 per cent. hydrochloric acid and 0·45 per cent. of solid matter. By this last treatment, a large amount of globulin was dissolved. As the digestive mixture would not filter through cloth it had to be neutralized directly with sodium hydroxide, which gave an abundant neutralization precipitate of so-called parapeptone, easily collected, and from which the fluid filtered perfectly clear. In order to obtain more material for study, the neutralization precipitate, together with the unaltered globulin, was treated a second time with 3 litres of the same active gastric juice for several days, by which the amount of globulin was reduced more than half, as shown by repeated neutralization.

The two neutralized, digestive fluids obtained in this manner, were alike in all respects; noticeably so in the remarkable fact that when weakly alkaline, they became turbid at 53° C., which turbidity increased as the solutions were heated to boiling. Furthermore, when made faintly acid, heat produced in both solutions an abundant flocculent precipitate which had the properties of coagulated albumin. This *coagulum from digested globulin* was purified by successive washings with boiling water, alcohol and ether. The following table shows the composition of the substance.

* See Zeitschrift für Biologie, Band xix, p. 184.

ANALYSIS OF THE COAGULUM FROM DIGESTED GLOBULIN.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·7229 | 0·4443 | 6·82 | 1·3667 | 51·55 | ---- | ---- | ---- | ---- | ----- | ---- | ----- | ---- |
| II | 0·4744 | 0·2950 | 6·90 | 0·8971 | 51·56 | ---- | ---- | ---- | ---- | ----- | ---- | ----- | ---- |
| III | 0·5634 | ----- | ---- | ----- | ---- | 75.0 | 18·8 | 765·2 | 15·75 | ----- | ---- | ----- | ---- |
| IV | 0·3546 | ----- | ---- | ----- | ---- | 47·43 | 19·6 | 764·2 | 15·76 | ----- | ---- | ----- | ---- |
| V | 0·6072 | ----- | ---- | ----- | ---- | ---- | ---- | ---- | ---- | 0·0753 | 1·70 | ----- | ---- |
| VI | 0·6474 | ----- | ---- | ----- | ---- | ---- | ---- | ---- | ---- | 0·0893 | 1·89 | ----- | ---- |
| VII | 0·7602 | ----- | ---- | ----- | ---- | ---- | ---- | ---- | ---- | ----- | ---- | 0·0073 | 0·96 |
| VIII | 0·6724 | ----- | ---- | ----- | ---- | ---- | ---- | ---- | ---- | ----- | ---- | 0·0060 | 0·89 |

The ash consisted mainly of $Fe_2O_3$.

*Percentage composition of ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·03 | 52·04 | ---- | ---- | ---- | ---- | *52·03* |
| H | 6·89 | 6·97 | ---- | ---- | ---- | ---- | *6·93* |
| N | ---- | ---- | 15·89 | 15·90 | ---- | ---- | *15·89* |
| S | ---- | ---- | ---- | ---- | 1·71 | 1·90 | *1·80* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *23·35* |
| | | | | | | | *100·00* |

### *Globulose Bodies.*

After removal of the above mentioned coagulum, the solution remained perfectly clear at all temperatures up to 100° C., even when rendered more strongly acid and also on subsequent neutralization. On the addition of nitric acid, however, to the cold solution, a precipitate was formed which disappeared on the application of heat, reappearing as the mixture became cool.

Crystals of salt alone, produced a heavy precipitate in the solution, while salt and acetic acid gave a still further precipitate, and when these reagents failed to cause any further precipitation, nitric acid or metaphosphoric acid would still give a noticeable turbidity.

In order to separate the globulose bodies from one another, the entire solution was concentrated on a water bath to the consistency of a thin syrup and then rubbed up in a mortar with salt in substance (the fluid being perfectly neutral), complete saturation being insured by long standing with an excess of salt crystals. The precipitate so formed being separated by filtration, the filtrate was partially precipitated by the cautious addition of 30 per cent. acetic acid saturated with salt, whereby a mixture of proto- and deutero-globulose was separated. After removal of this precipitate the filtrate was finally treated with more of the above acetic acid until nothing further was precipitated.

The various precipitates were then subjected to strong pressure to remove as much of the salt-saturated fluid as possible, then dissolved in water and dialyzed for the complete removal of the salt and to separate heteroglobulose.

### *Protoglobulose.*

This body, precipitable by sodium chloride alone, was purified by saturating the first dialyzed solution again with salt, then dialyzing a second time under repeated changes of reaction, by the alternate addition of acetic acid and sodium carbonate and finally by neutral reaction, until all chlorine was removed from the solution and the admixed heteroglobulose completely separated. The clear filtered fluid was then concentrated, after which the protoglobulose was precipitated with alcohol, washed with alcohol and ether and so obtained as an almost white powder. The substance, so prepared, gave when rubbed up with cold water, a filtrate not quite clear and with a noticeably alkaline reaction. It differed from a solution of proto-albumose from fibrin in one respect, viz: that on boiling in the presence of a small amount of sodium chloride it became quite turbid,

the turbidity, however, disappearing completely as the solution became cool, (the opposite of the albumose reaction : compare Zeitschrift für Biologie, Band xx, p. 45). We think it may be assumed that this single deviation from the reactions of fibrin-protoalbumose is not due to the presence of impurities (heteroglobulose, etc.), because no heteroglobulose whatever separated from a portion of the sample, even after dialyzing a week longer. If the solution was made even very slightly acid or alkaline, the turbidity did not then occur on heating. Further, the small content of ash (0·4 per cent.), which consisted only of calcium sulphate with a trace of ferric oxide, testifies to the purity of the preparation and the completeness of the dialysis. The preparation was analyzed with the results shown in the accompanying table.

*Deuteroglobulose.*

This body is about as difficult to purify from the preceding one as deuteroalbumose from protoalbumose. We succeeded, however, in separating it by rejecting the first portions of the precipitate produced by acetic acid and sodium chloride and using only the last portions precipitated; or after the acetic acid failed to give any further precipitate, by using the small precipitate produced by the moderate addition of alcohol. This last precipitate naturally enclosed considerable sodium chloride, but the deuteroglobulose was obtained perfectly pure after removing the salt by dialysis, since the globulose solution, even when noticeably acid, gave no turbidity whatever on the addition of salt in substance. The quantity, however, was unfortunately too small for analysis. It sufficed only for determining the reactions, which agreed with those of the substance obtained by the later precipitation with acetic acid except in one particular, viz: that the latter preparation in a neutral or slightly alkaline solution showed an extremely slight turbidity on the addition of crystals of sodium chloride. All other reactions were identical and corresponded so completely with those of deuteroalbumose that it is only necessary to call attention to the latter (see Zeitschrift für Biologie, Band xx, pp. 26–28, or Amer. Chem. Jour., vol. vi, pp. 46–47) and to especially mention the non-precipitation of deuteroglobulose in a solution free from salt, by nitric acid in any quantity and at any temperature.

Since no heteroglobulose whatever separated from the solution of the last acetic acid precipitate during dialysis, even with repeated change of reaction, the substance was therefore prepared for analysis,

ANALYSIS OF PROTOGLOBULOSE.

| No. | Substance used. gram. | $H_2O$ found. gram | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. ° C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | Ash found gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·6707 | 0·4211 | 6·97 | 1·2577 | 51·13 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·4926 | 0·3070 | 6·92 | 0·9300 | 51·48 | ---- | ---- | ---- | ---- | --- | ---- | ---- | ---- |
| III | 0·5328 | ---- | ---- | ---- | ---- | 72·3 | 18·8 | 763·56 | 16·02 | ---- | ---- | ---- | --- |
| IV | 0·4313 | ---- | ---- | ---- | ---- | 59·23 | 19·9 | 758·14 | 16·03 | ---- | ---- | ---- | ---- |
| V | 0·7436 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·1188 | 2·19 | ---- | ---- |
| VI | 0·5765 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0924 | 2·20 | ---- | ---- |
| VII | 0·9137 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0043 | 0·47 |
| VIII | 0·5124 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0025 | 0·48 |
| IX | 0·3350 | 0·2100 | 6·96 | 0·6308 | 51·34 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |

*Percentage composition of ash-free Protoglobulose.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 51·38 | 51·78 | 51·59 | ---- | ---- | ---- | ---- | *51·57* |
| H | 7·00 | 6·95 | 6·99 | ---- | --- | ---- | ---- | *6·98* |
| N | ---- | ---- | ---- | 16·09 | 16·10 | ---- | ---- | *16·09* |
| S | ---- | ---- | ---- | ---- | ---- | 2·20 | 2·21 | *2·20* |
| O | --- | ---- | ---- | --- | ---- | ---- | ---- | *23·16* |
| | | | | | | | | *100·00* |

simply by concentration of the solution freed from chlorine, precipitation with alcohol and washing with ether. The ash (1·17 per cent.) consisted only of calcium phosphate and a trace of sulphate.

### *Heteroglobulose.*

This body was obtained from the gummy precipitate which separated, during dialysis, from the solution of the first precipitate thrown down from the neutralized digestive fluid by salt alone. The sticky mass was separated from the sides of the parchment tubes, dissolved in sodium chloride of from 3 to 5 per cent., reprecipitated by saturation of the solution with salt, the precipitate again dissolved in dilute salt solution and the substance finally separated by long continued dialysis in running water. After thorough washing with water, alcohol and ether it appeared as a light, white powder, not unlike heteroalbumose in general behavior and reactions. After each precipitation and treatment with dilute sodium chloride, heteroglobulose left a residue, which like dysalbumose was readily soluble only in dilute acids. From the following analysis it is to be seen that the preparation, in spite of its long continued and repeated dialysis, contained 2·03 per cent. of ash, which consisted mainly of calcium carbonate with a small amount of phosphate and sulphate.

The composition of the three globulose bodies shows the same slight differences as noticed in the case of the various albumose bodies (from fibrin). Unlike the latter, however, the content of carbon in the globulose bodies never falls below 51 per cent., and furthermore it is always higher than that of the globulin from which the globulose was derived. The percentage of nitrogen, which in the albumose bodies was found a little higher than in fibrin, exceeds that of the globulin more yet, in some cases by more than 1 per cent., and the same holds true of the percentage of sulphur. In contrast to the albumose bodies, the percentage composition of the globulose bodies gives no grounds whatever for the assumption that they arise from the digested globulin by simple hydration. It must not be forgotten, however, that the digestion of globulin by gastric juice is a process quite different from that of fibrin digestion and one hitherto much less clearly understood, since besides the globulose bodies there is formed a large quantity of a substance which is separated by boiling and which resembles ordinary coagulated albumin. Something similar, indeed, has been known ever since Brücke's study of fibrin digestion, but it has long been accepted that the coagulum

ANALYSIS OF DEUTEROGLOBULOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | Ash found. gram, | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4711 | 0·2910 | 6·86 | 0·8790 | 50·88 | .... | .... | .... | .... | .... | .... | .... | .... |
| II | 0·3468 | 0·2149 | 6·88 | 0·6481 | 50·96 | .... | .... | .... | .... | .... | .... | .... | .... |
| III | 0·5403 | .... | .... | .... | .... | 72·94 | 19·6 | 756·10 | 15·73 | .... | .... | .... | .... |
| IV | 0·5106 | .... | .... | .... | .... | 69·01 | 19·4 | 756·10 | 15·76 | .... | .... | .... | .... |
| V | 0·4616 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0656 | 1·95 | .... | .... |
| VI | 0·5634 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0693 | 1·69 | .... | .... |
| VII | 0·6472 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0893 | 1·89 | .... | .... |
| VIII | 0·6540 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 0·0076 | 1·16 |
| IX | 0·6447 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 0·0077 | 1·19 |

*Percentage composition of ash-free Deuteroglobulose.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 51·48 | 51·56 | .... | .... | .... | .... | .... | *51·52* |
| H | 6·94 | 6·96 | .... | .... | .... | .... | .... | *6·95* |
| N | .... | .... | 15·92 | 15·95 | .... | .... | .... | *15·94* |
| S | .... | .... | .... | .... | 1·97 | 1·71 | 1·91 | *1·86* |
| O | .... | .... | .... | .... | .... | .... | .... | *23·73* |
| | | | | | | | | *100·00* |

ANALYSIS OF HETEROGLOBULOSE.

| No. | Substance used gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c c. | N found. T. ° C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5200 | 0·3187 | 6·80 | 0·9745 | 51·10 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·3475 | 0·2149 | 6·87 | 0·6500 | 51·00 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·3625 | ---- | ---- | ---- | ---- | 48·44 | 20·0 | 764·74 | 15·73 | ---- | ---- | ---- | ---- |
| IV | 0·3438 | ---- | ---- | ---- | ---- | 46·07 | 19·6 | 764·57 | 15·79 | ---- | ---- | ---- | ---- |
| V | 0·5227 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0822 | 2·15 | ---- | ---- |
| VI | 0·6045 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0916 | 2·08 | ---- | ---- |
| VII | 0·5811 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0120 | 2·06 |
| VIII | 0·4897 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0098 | 2·00 |

*Percentage composition of ash-free Heteroglobulose.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52.15 | 52·05 | ---- | ---- | ---- | ---- | *52·10* |
| H | 6·95 | 7·02 | ---- | ---- | ---- | --- | *6·98* |
| N | ---- | ---- | 16·05 | 16·11 | ---- | ---- | *16·08* |
| S | ---- | ---- | ---- | ---- | 2·20 | 2·13 | *2.16* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·68* |
| | | | | | | | *100·00* |

obtained by Brücke on boiling the neutralized digestive fluid, arose from the globulin present in the fibrin employed, which had not been previously washed with salt water. Globulin, moreover, yields this body in much greater quantity, even after several days' exposure to the action of an energetic gastric juice and it was still found abundantly among the products of a second digestion of the first neutralization precipitate.

*Comparison of the Analyses.*

| | Globulin. | Coagulum from digested Globulin. | Proto-globulose. | Deutero-globulose. | Hetero-globulose. | | Hemialbumose from urine* (Osteomalachia). | Hetero-albumose from Fibrin.† | Fibrin ‡ |
|---|---|---|---|---|---|---|---|---|---|
| C | 51·14 | 52·03 | 51·57 | 51·52 | 52·10 | C | 52·13 | 50·88 | 52·68 |
| H | 7·00 | 6·93 | 6·98 | 6·95 | 6·98 | H | 6·83 | 6·89 | 6·83 |
| N | 14·64 | 15·89 | 16·09 | 15·94 | 16·08 | N | 16·55 | 17·08 | 16·91 |
| S | 1·67 | 1·80 | 2·20 | 1·86 | 2·16 | S | (1·09?) | 1·23 | 1·10 |
| O | 25·55 | 23·35 | 23·16 | 23·73 | 22·68 | O | 23·40 | 23·92 | 22·48 |

In this review of the composition of globulin and of the products of its digestion we have included also an analysis of fibrin, of a fibrin-albumose and of hemialbumose from the urine of a person with osteomalachia.

We call attention again to the latter because its surprising correspondence, especially to heteroglobulose, appears to confirm the belief, expressed in our former paper that the difference in the albumose from urine and that from fibrin depends on the formation of the former from an albuminous body, whose digestion, at least as regards the formation of albumose bodies, was then unknown, and for which we had already turned to globulin.

In order to gain further information concerning the cleavage of globulin in the process of digestion, the remaining material was used in the following experiments.

1. Heteroglobulose dissolved in 0·3 per cent. sodium carbonate and warmed at 40° C. for fourteen days with pure trypsin (with the addition of thymol, as usual) remained perfectly clear, even after neutralization, and failed to yield afterwards any body resembling antialbumid. Among the products of the digestion, there was found in addition to an abundance of antipeptone, only a trace of leucin, no tyrosin whatever, while with bromine water the alcoholic extract,

* Compare Zeitschrift für Biologie, Band xix, p. 202. † Ibid., Band xx, p. 40.

‡ According to Hammarsten.

which had been dissolved in water after driving off the alcohol, became simply a little darker, but not rose-colored or violet. Hence heteroglobulose is to be considered as belonging to the anti group.

2. Protoglobulose, which still contained some heteroglobulose, when treated in the same manner with trypsin, behaved similarly but afforded besides an abundance of leucin, also some tyrosin and an extract which became deep violet on the addition of bromine water. Hence protoglobulose gives evidence of belonging to the hemi group.

Finally, we submitted to the digestive action of trypsin the third and fifth precipitates (so-called parapeptone) which were separated in continually decreasing quantities by neutralization, after renewed, energetic pepsin digestion of the original globulin. Both failed to yield any coagulum during their digestion with 0·3 per cent. sodium carbonate, and after the trypsin had acted for fourteen days, neutralization with acetic acid yielded a heavy precipitate, while considerable antipeptone was found in the solution. Although the digestion of the third neutralization precipitate still afforded a trace of leucin and tyrosin without giving any reaction with bromine, no leucin, tyrosin or a substance colored by bromine water could be obtained from the precipitate separated after the fifth pepsin digestion.

Hence globulin, like fibrin and other albuminous bodies, yields during pepsin digestion at the last only bodies of the anti group, which are peptonized, though slowly, by trypsin, but yield no further cleavage products.

## XV.—Peptones. By W. Kühne and R. H. Chittenden.

Since there has been discovered in neutral ammonium sulphate a means for the complete precipitation of the albumose bodies, we have been induced to take up anew our former investigations on the behavior and composition of peptones. As these latter bodies are not precipitated by the ammonium salt, we had expected to obtain peptones free from the primary cleavage products of albumin and thereby advance another step in our knowledge of the definite products of the proteolytic action both of pepsin and of trypsin. Renewed investigation was demanded by the probability that hitherto pepsin-peptones entirely free from albumose have never been obtained, for such peptones as are to be found in commerce or in the hands of the most careful investigator of gastric digestion can readily be shown to contain albumose by saturating a solution of the preparation with ammonium sulphate. There will result an abundant precipitate of albumose and a surprisingly small residue of non-precipitated peptones or the entire absence of such a residue. Only antipeptone obtained by trypsin digestion will occasionally form an exception, and even then in most cases we cannot but doubt that the peptones so formed are wholly free from albumose.

In order to be certain of the presence of peptones in a digestive fluid, it must be made slightly acid with acetic acid, rubbed up with ammonium sulphate till saturated and then filtered from the excess of salt and the albumose precipitate. If the filtrate is thereupon treated with a large excess of strong sodium hydroxide and then a few drops of very dilute cupric sulphate be added, the appearance of the rosy red color of the biuret reaction will indicate the presence of peptones. If peptones are absent the fluid will be pure blue without a tinge of violet, since the solution can contain no other albuminous body. Even after an apparently energetic pepsin digestion the latter result is not at all rare, and a heavy precipitate by the ammonium salt is so frequently seen, that it is still to be doubted whether there is a pepsin-acid digestion which causes the disappearance of all albumose. On the contrary, the albumose precipitate after a sufficiently long and energetic trypsin digestion is very slight and peptone is to be found abundantly in the solution.

We have endeavored to prepare pure peptones in quantity from the solution saturated with ammonium sulphate. For this purpose the solution was first freed from the greater part of the salt by concentration and crystallization. During this process a small amount of a nitrogenous substance separated, perhaps albumose formed again from peptones when the solution was vigorously boiled and the temperature rose to 110° C. The mother liquor, after suitable dilution, was boiled with hot saturated baryta water until all ammonia was expelled, during which operation the precaution was taken to use no excess of barium hydroxide and thus decompose the peptones.

From time to time, therefore, portions were filtered, tested for sulphate, and when this became small in amount the last portions of sulphuric acid were removed by barium carbonate. From the filtrate, which always contained much barium, the latter was entirely removed by dilute sulphuric acid, either immediately or after a previous purification of the barium peptone compound. The peptones were then precipitated with alcohol and occasionally further purified with phosphotungstic acid. Naturally the large amount of ammonium sulphate to be removed formed a correspondingly troublesome quantity of barium sulphate, which could be handled only in large filtering bags, and occasioned a large loss of peptone in spite of a most careful washing of the precipitate with boiling water and the application of pressure. On evaporating the peptone solution, which contained but little salt, no resinous precipitate resembling albumose was to be seen.

### 1. *Amphopeptone.*

We have designated as amphopeptone the end product of the digestion of albumin by pepsin and acid. The first attempt to obtain this peptone free from albumose and in a quantity in some degree proportionate to our wants, showed us that there was needed not only the most active digestive fluid possible and long exposure to a temperature of 40° C. but also a very large amount of pepsin. Such a quantity of the ferment could be procured, however, only by first dissolving considerable quantities of the mucous membrane of the stomach in acid; quantities which must be taken into consideration, in addition to the fibrin to be digested, since something is formed in the self-digestion of the mucous membrane which necessarily remains mixed with the peptone. It is known that mistakes have already been committed by not distinguishing the products arising from the material of the mucous membrane, from those derived from the digested substance. For example, Hoppe-Seyler's erroneous asser-

tion that pepsin digestions yield leucin and tyrosin, rests wholly upon this circumstance, for since the digestion of the mucous membrane always commences with the disappearance of a mucilaginous substance, the derivatives of the latter must necessarily be expected in the resultant solution. Probably for this reason, artificial gastric juice which has been prepared from mucous membrane and is no longer mucilaginous, gives a precipitate when treated with alcohol which differs much from the precipitates of albumose and peptone in being almost as elastic as rubber and, as a rule, forming when shaken, a single ball in which the pepsin is then ordinarily inclosed. We have not yet examined this substance closely, since in the course of the investigations to be described, another more suitable method for precipitating and isolating the ferment has been discovered. We shall designate this elastic body for convenience, mucin-peptone. This mucin-peptone might possibly conceal the whole amount of peptone expected from the digested fibrin, or remain mixed with the latter in considerable quantity. In spite of this objection, which we at no time lost sight of, we prepared a quantity of fibrin-peptone without attempting to remove or to prevent the mixture in question. The observations made by Dr. Pollitzer* in the Physiological Institute at Heidelberg, on the influence of pepsin-peptone free from albumose on coagulation of the blood, were performed with such amphopeptone, which is not perfectly pure.

Supported by the following analyses of this peptone in our presumption that it was rendered impure by mucin-peptone, we sought a process that would exclude this impurity. This was found almost of itself after we had noticed that ammonium sulphate invariably precipitated from the acid solutions, in addition to albumose, the entire quantity of active pepsin. While, therefore, nothing capable of digestion with acids could in any way be obtained from the filtrates, an exceedingly active juice was formed by dissolving the precipitate in dilute hydrochloric acid. Hereafter, we accordingly prepared the strong pepsin solution, by simply precipitating large quantities of very concentrated gastric juice containing 0·5 per cent. hydrochloric acid with ammonium sulphate and dissolving the resinous precipitate, which did not contain an objectionable quantity of albumose bodies, in fresh dilute acid. By this means the mucin-peptone was gotten rid of, since it could not be precipitated by ammonium sulphate and thus a new method was found for preparing and isolating pepsin, which we shall enter upon at another time.

* Verhandl. d. Naturhist. med. Verein zu Heidelberg, N. F. III, p. 293.

1. *Amphopeptone prepared with ordinary gastric juice.*

Gastric juice, prepared from 145 grams of isolated mucous membrane from the fundus of pigs' stomachs by two days self-digestion in two litres of 0·4 per cent. hydrochloric acid, was added to 585 grams of well washed and boiled fibrin, previously swollen in four litres of acid of the same strength and the whole warmed for two days more at 40° C. The thin fluid-like mixture so obtained, was neutralized with sodium hydroxide and then filtered from the undissolved residue of the mucous membranes (nuclei of the gland cells) and the slight neutralization precipitate. After being made slightly acid with acetic acid, the fluid was heated to boiling, evaporated to two litres, then saturated with neutral ammonium sulphate, separated from the slight coagulum and precipitated albumose, again concentrated to one litre and freed from a large portion of the ammonium sulphate by crystallization at 0° C. In order to still further separate the salt, the solution was treated with one litre of absolute alcohol, again placed in the cold, and finally strained through linen to remove the fine powdery salt, which was wholly free from precipitated peptone. After having been freed from alcohol, by vigorous boiling and concentration to the consistency of syrup and from much salt by crystallization, the thick fluid was filtered by suction, boiled after much dilution with a large amount of barium carbonate until the odor of ammonia had vanished. From the solution, separated from the barium sulphate and again much concentrated, alcohol precipitated the peptone as a barium compound which could be freed from salts (especially sodium chloride) by repeated precipitation and boiling with alcohol. Finally the barium-peptone was decomposed as much as possible with dilute sulphuric acid. As was seen later from the concentrated peptone solution, there remained dissolved a trace of sulphuric acid, but only enough to make the fluid assume a slight opalescence after boiling with barium chloride and hydrochloric acid. An attempt was made to purify the isolated peptone by evaporating, precipitating with alcohol, dissolving in water and reprecipitating with alcohol. This did not succeed well, as shown later by the high percentage of ash. By drying first on a water bath, then in an air bath at 105° C. with frequent stirring, which destroyed the firm resinous surface, the peptone gradually became solid, and changed to a puffed up mass. The resulting product could be ground, when cold, to a light, very hygroscopic powder and weighed in this condition 25 grams.

In an attempt to dry the substance for analysis, during which the temperature was allowed to rise to 110° C. it was found impossible to obtain a constant weight, perhaps on account of decomposition setting in, as suggested by an unpleasant odor which had begun to develop while on the water bath. Portions of 8–10 grams lost daily 0·03–0·04 gram. The analyses were accordingly made only after drying many days. Portions purified with alcohol, dissolved in boiling water with addition of hydrochloric acid, gave no reaction to be distinguished when heated with barium chloride.

Carbon, hydrogen and nitrogen were determined as before, the sulphur by a method already used by us to some extent,* viz: by fusion with potassium hydroxide and potassium nitrate according to the method distinguished by Hammarsten as 1*a*.†

The results of the analysis (Amphopeptone A), shown by the following table, were hardly satisfactory and the low percentage of carbon, particularly, was quite a surprise to us, hence we proceeded at once to the previously mentioned preparation of a peptone, which would probably be rendered less impure by derivatives of the mucous membrane and which would, moreover, be easier to purify further.

### 2. *Amphopeptone prepared with purified pepsin.*

*Preparation of the pepsin.*—1220 grams of isolated mucous membrane from the fundus of ten pigs' stomachs were warmed at 40° C. with seven litres of 0·5 per cent. hydrochloric acid for six days. The mixture was then saturated directly with ammonium sulphate, by which a resinous precipitate, with large, sticky lumps was formed, easily collected on a cloth filter. After pressing out the salt solution as much as possible and washing with water, the gummy mass was dissolved in five litres of 0·4 per cent. hydrochloric acid and warmed again at 40° C. for a few days. Then for the first time it was filtered through paper. As preliminary experiments had shown that gastric juice which contains small quantities of ammonium sulphate molds easily, the second digestion and the following fibrin digestion were carried on in the presence of 0·25 per cent. of thymol, which wholly prevented the formation of mold. The mass submitted for the second time to self-digestion, gave now with the ammonium salt a much smaller precipitate, which contained only a very

* Compare our earlier papers. Zeitschrift für Biologie, vols. xix and xx.

† Zeitschrift für physiol. Chem., vol. ix, p. 288.

AMPHOPEPTONE (A).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash % | $BaSO_4$ from the ash. gram. | S from ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5615 | 0·3020 | 6·05 | 0·8406 | 40·82 | ---- | -- | ---- | ---- | ---- | ---- | ----- | ---- | ---- | ----- | ---- |
| II | 0·4269 | 0·2267 | 5·90 | 0·6420 | 41·01 | ---- | -- | ---- | ---- | ---- | ---- | ----- | ---- | ---- | ----- | ---- |
| III | 0·5601 | 0·3005 | 5·96 | 0·8410 | 40·95 | ---- | -- | ---- | ---- | ---- | ---- | ----- | ---- | ---- | ----- | ---- |
| IV | 0·5622 | ----- | ---- | ----- | ---- | 70·0 | 4·2 | 757·1 | 15·34 | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| V | 0·4042 | ----- | ---- | ----- | ---- | 50·2 | 4·6 | 764·2 | 15·42 | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| VI | 0·5174 | ----- | ---- | ----- | ---- | ---- | -- | ---- | ---- | 0·0426 | 8·23 | ----- | ---- | ---- | ----- | ---- |
| VII | 0·5625 | ----- | ---- | ----- | ---- | ---- | -- | ---- | ---- | 0·0450 | 8·00 | ----- | ---- | ---- | ----- | ---- |
| VIII | 0·4930 | ----- | ---- | ----- | ---- | ---- | -- | ---- | ---- | ----- | ---- | 0·0295 | 0·82 | 0·59 | ----- | ---- |
| IX | 9·7954 | ----- | ---- | ----- | ---- | ---- | -- | ---- | ---- | ----- | ---- | 0·0550 | 0·94 | 0.71 | ----- | ---- |
| X | 1·0799 | ----- | --- | ----- | ---- | ---- | -- | ---- | ---- | ----- | ---- | ----- | ---- | ---- | 0·0183 | 0·23 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 44·42 | 44·62 | 44·55 | ---- | ---- | ---- | ---- | *44·53* |
| H | 6·58 | 6·42 | 6·48 | ---- | ---- | ---- | ---- | *6·49* |
| N | ---- | ---- | ---- | 16·69 | 16·78 | ---- | ---- | *16·73* |
| S | ---- | ---- | ---- | ---- | ---- | 0·66 | 0·78 | *0·72* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *31·53* |
| | | | | | | | | *100·00* |

small amount of albumose bodies, while a portion digested as a test for the third time, gave in the filtrate from the precipitate produced by the ammonium salt, so faint a biuret reaction for peptones that it was plainly evident, that the slight residue of albumins from the mucous membrane now remaining, could be overlooked without danger.

*Digestion of the fibrin.*—3800 grams of washed but not boiled fibrin were digested with the twice precipitated pepsin, which was dissolved in ten litres of 0·4 per cent. hydrochloric acid. To obtain as little albumose and as much peptone as possible, the mixture was allowed to remain at 37°–40° C. for two weeks. At the end of that time, filtered portions gave only slight precipitations by neutralization, but a heavy precipitate was obtained with ammonium sulphate, with sodium chloride, with sodium chloride and acetic acid, and still further by sodium chloride and nitric acid or metaphosphoric acid. Nevertheless the filtrate saturated with ammonium sulphate contained much peptone.*

*Preparation and purification of the peptone.*—For this purpose the filtrate was neutralized with sodium hydroxide, filtered through linen, especially for removing the impurities of the fibrin, the filtrate slightly acidified with acetic acid, concentrated to about four litres, precipitated with an excess of ammonium sulphate, filtered and pressed, the solution boiled with barium hydroxide and finally with barium carbonate and a large quantity of water, until ammonia could no longer be detected. The barium sulphate was then removed by filtration through cloth bags which were repeatedly washed and pressed, the solution evaporated to about four litres, the barium-peptone decomposed with a very slight excess of sulphuric acid, the new precipitate of barium sulphate filtered off, the solution concentrated to two litres, the free acid neutralized with ammonia and after cooling, six per cent. English sulphuric acid (previously diluted) was added; then the sulphuric acid-peptone solution was precipitated with a large excess of phosphotungstic acid, the precipitate washed first with six per cent. sulphuric acid, then with a large quantity of water, after which the compound was decomposed with excess of barium hydroxide and the excess completely removed from the fil-

* Later experiments have shown that pepsin acts much more energetically if the ammonium sulphate is completely removed by dialysis, before each new solution and digestion of the pepsin-containing precipitate in hydrochloric acid, and further, that *nearly pure pepsin* becomes wholly inactive by being warmed with dilute hydrochloric acid in the presence of even small quantities of ammonium sulphate.

trate with sulphuric acid. The peptone solution thus obtained had a distinctly acid reaction, and strange to say, contained hydrochloric acid, which was hardly to be expected after the very careful washing which the precipitate had received. The solution was neutralized with ammonia to render the acid harmless on concentration. Then we succeeded in obtaining the evaporated residue free from ammonium chloride by repeated precipitation and boiling with alcohol.

As already mentioned, the method gives rise to much loss and the same holds true of the otherwise excellent precipitation of peptone by phosphotungstic acid according to the method of Hofmeister, for so far as our experience extends, peptones cannot be *completely* precipitated in this manner. There arises in the filtrate, containing excess of phosphotungstic acid, not only additional turbidity and precipitation due to peptone, but considerable quantities of peptone are still found in the liquid, which has perhaps remained clear for months, if treated with barium hydroxide;—a circumstance which we were not able to prevent even by strongly acidifying the solution to be precipitated with phosphotungstic acid, with either sulphuric or hydrochloric acid.

*Behavior of the peptone.*—This peptone was also difficult to convert into a dry state, although we did succeed ultimately in bringing it to a constant weight as a fine, exceedingly hygroscopic powder, by heating for some time at 105° C. *in vacuo.* The first difficulty was found in commencing the drying, for although we treated the glue-like mass repeatedly with absolute alcohol, then for a long time with ether and finally boiled it again with alcohol, thereby changing it into an almost dry, crumbling condition, we were compelled at last to stop its further direct drying, since at 100° C. the preparation took on the consistency of pitch and formed a bulky foam from which alcohol vapor continually escaped. Therefore the alcohol was first driven out by thorough boiling with water and the latter removed as much as possible at 100° C. This was also a tedious performance for, although the substance no longer foamed up so violently, it did not become dry until after many days of stirring and breaking the covering which continually bubbled up. The same proceeding was repeated, although in a less degree, on transferring the substance to the air bath at 105° C., and only the single portions taken for analysis could be brought to a constant weight without puffing up further at 105° C.

While drying, the unpleasant odor noticed from amphopeptone A was also observed here, although only in a slight degree.

The peptone thus obtained appears (when dried at 105° C.) as a dry, light yellowish powder. It can be preserved in this form only when most tightly stoppered. In the air it soon forms large balls, becomes sticky like pitch and melts to a tough mass which does not become visibly thinner. What is truly surprising is the behavior of the peptone towards water. *A bit of the powder wet with a small drop of water hisses and steams like phosphoric anhydride when moistened, and when this, or the powdered but not absolutely dry preparation which no longer hisses, is dissolved in water, a development of heat is to be noticed.* We have observed the same remarkable peculiarity in anti-peptone to be described later.

Analysis of the preparation (Amphopeptone B) dried at 105° C. *in vacuo*, gave the results tabulated in the accompanying table.

### *Amphopeptone (b).*

After these results were obtained, an attempt was made to reduce the ash content of the preparation by repeated precipitation with alcohol, which succeeded so well that the substance when dried *in vacuo* over sulphuric acid, later at 106° C. until of constant weight, contained then 2·15 per cent. of ash instead of 3·25 per cent.

I. 0·5500 gram of this preparation gave 0·3400 gram $H_2O$ = 6·86 per cent. H and 0·9566 gram $CO_2$ = 47·43 per cent. C.

II. 0·7121 gram of substance gave 98·4 c. c. N at 16·4° C. and 765·5mm. pressure = 16·49 per cent. N.

III. 0·7839 gram substance gave 0·0169 gram ash = 2·15 per cent. of ash.

Therefore in the ash-free substance (Amphopeptone b) there are—

48·47% C, 7.02% H, 16·86% N.

## II. *Antipeptone.*

As with amphopeptone, we have formed in various digestion experiments several preparations of antipeptone by the action of trypsin. These preparations have been studied both after purification with alcohol and after further purification with phosphotungstic acid, but in every case after complete removal of the albumose bodies. We have not attempted to meet the objection that the antipeptone is not formed exclusively from the digested fibrin, but in part from the albumins of the pancreas. This would have necessitated experiments with pure trypsin, which seemed too costly to undertake. Instead of this we have studied a peptone which we shall distinguish

AMPHOPEPTONE (B).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. C. C. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash % | $BaSO_4$ from the ash. gram. | S from the ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·8347 | 0·5820 | 7·08 | 1·4379 | 46·97 | ---- | --- | ---- | ---- | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| II | 0·3511 | 0·2176 | 6·88 | 0·6097 | 47·35 | ---- | --- | ---- | ---- | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| III | 0·4170 | ----- | ---- | ----- | 47·21 | ---- | --- | ---- | ---- | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| IV | 0·4694 | ----- | ---- | ----- | ---- | 63·56 | 21.2 | 758·2 | 15·74 | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| V | 0·6948 | ----- | ---- | ----- | ---- | 93·88 | 21·0 | 758·8 | 15·75 | ----- | ---- | ----- | ---- | ---- | ----- | ---- |
| VI | 0·6452 | ----- | ---- | ----- | ---- | ---- | --- | ---- | ---- | 0·0210 | 3·25 | ----- | ---- | ---- | ----- | ---- |
| VII | 0·6712 | ----- | ---- | ----- | ---- | ---- | --- | ---- | ---- | 0·0215 | 3·20 | ----- | ---- | ---- | ----- | ---- |
| VIII | 0·5032 | ----- | ---- | ----- | ---- | ---- | --- | ---- | ---- | ----- | ---- | 0·0360 | 0·98 | 0·68 | ----- | ---- |
| IX | 0·5936 | ----- | ---- | ----- | ---- | ---- | --- | ---- | ---- | ----- | ---- | 0·0477 | 1·10 | 0·80 | ----- | ---- |
| X | 1·3164 | ----- | ---- | ----- | ---- | ---- | --- | ---- | ---- | ----- | ---- | ----- | ---- | ---- | 0·0288 | 0·30 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 48·54 | 49·93 | 48·79 | ---- | ---- | ---- | ---- | *48·75* |
| H | 7·31 | 7·11 | ---- | ---- | ---- | ---- | ---- | *7·21* |
| N | ---- | ---- | ---- | 16·26 | 16·27 | ---- | ---- | *16·26* |
| S | ---- | ---- | ---- | ---- | ---- | 0·71 | 0·83 | *0·77* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *27·01* |
| | | | | | | | | *100·00* |

as *gland peptone*, being derived exclusively from the self-digestion of the albuminous bodies of the gland substance, without any addition of fibrin or other albumin.

How superior the action of trypsin is to that of pepsin, is seen not alone in the total decomposition of hemipeptone which is accomplished only by the former, but also in the incomparably more rapid and perfect change of albumose to peptone. Hence the particularly troublesome and tedious treatment of antipeptone with ammonium sulphate may well seem superfluous when there is no albumose present. We must however be perfectly sure, by a preliminary test with that salt, that albumose is absent and its use is unavoidable where impure trypsin is employed in large quantities; that is, where an infusion of the pancreatic gland or the so-called artificial pancreatic juice is used. From the latter, ammonium sulphate precipitates a mixture which contains besides unaltered, highly active trypsin, whose isolation we propose later to study in this way, various other bodies, such as albumose, whose removal is necessary in the preparation of pure peptone.

## *Antipeptone* (C).

*Preparation of the pancreatic juice.*—100 grams of dried ox pancreas, purified with alcohol and ether, were warmed at 40° C. with 500 c. c. of 0·1 per cent. salicylic acid for 12 hours, and filtered through muslin. The residue was then mixed with 500 c. c. of 0·25 per cent. sodium carbonate, a little thymol added and the mixture again warmed at 40° C. for 12 hours. The acid solution, after it had been neutralized, was brought to the same degree of alkalinity with sodium carbonate, a little thymol added and also warmed at 40° C. for the same length of time. After filtering and pressing the residue of tissue, both filtrates were united. The weight of the undissolved residue, dried at 100° C., amounted as usual to 12 grams. Thus the nuclei of the cells, the collagen and the portion of elastin undigested under these conditions, is equal to 12 per cent. of the dry pancreas, freed from fat.

*Digestion of the fibrin.*—300 grams of dry fibrin, purified by washing and boiling with water, then with alcohol and finally by extraction with ether were softened with boiling water (when the weight amounted to 970 grams after squeezing with the hands), then warmed at 40° C. with 3 litres of 0·25 per cent. sodium carbonate containing 0·5 per cent. of thymol. To this was added the whole

infusion obtained from the 88 grams of self-digested pancreas, after which the mixture was continued at 40° C. for six days. At the end of the first day nearly all of the fibrin had disappeared, although a considerable portion appeared to float on the surface of the fluid. When examined more closely, however, this residue proved to be extremely light, hollow, easily crushed and with a somewhat greasy feeling. A similar residue, the amount of which we did not determine, remained at the end of six days and consisted mainly of antialbumid with much tyrosin.

*Preparation of the antipeptone.*—The solution resulting from the above digestion was made slightly acid with acetic acid, boiled, passed through a filtering bag, concentrated to 1 litre, and freed from a large amount of leucin and tyrosin by crystallization and filtration. The resultant, brownish-looking syrup was treated with alcohol until peptones began to precipitate, and after the latter had been redissolved by boiling the solution, it was placed aside for crystallization. The filtrate, which now contained only a small amount of amido acids, was freed from alcohol by boiling, diluted with a saturated solution of ammonium sulphate, which had also served for washing out the mass of crystals on the filters, and then completely saturated with the ammonium salt in substance. After separating the slight precipitate so formed, in which some leucin and tyrosin was detected, the greater portion of the ammonium salt was removed from the filtrate by repeated concentration and crystallization, while the remainder was gotten rid of, as before, with barium hydroxide and barium carbonate. Since in this case, precipitation with phosphotungstic acid could not yet be employed, we attempted to purify the peptone as much as possible from other products of digestion (amido acids), first, as a barium compound by repeated precipitation and boiling with alcohol, after which the barium-peptone was exactly decomposed with sulphuric acid and the free peptone purified in a similar manner by repeated precipitation and extraction with alcohol, once or twice in the presence of a little acetic acid. The peptone thus obtained, when dried at 105° C., weighed 120 grams. Assuming that albuminous bodies by complete typsin digestion, split up into 50 per cent. of products arising from the wholly decomposable hemipeptone and 50 per cent. of antipeptone not further changed by typsin, then the amount obtained—120 grams—agrees with this so far as it is possible, with the unavoidable losses which the treatment of large quantities in this manner implies. The 388 grams of dry albumin (300 grams of fibrin and 88 grams of self-

digested material from the pancreas) would have had to yield 194 grams of antipeptone if there were no loss. The loss, however, of 74 grams noticed in our experiment is sufficiently explained by the noticeable solubility of peptone in the water contained in alcohol, and by the conversion of a portion of the peptone into antialbumid.

*Behavior of the antipeptone.*—This peptone was still more difficult to dry than the amphopeptone formed by pepsin digestion, and it could only be accomplished after the removal of all alcohol by thorough boiling with water. As the solution became very concentrated on the water-bath, hydrogen sulphide, as shown by reaction with lead acetate, was given off together with a strong odor of valerianic acid, which was also evolved quite noticeably at 105° C. In order to obtain a constant weight it was necessary to dry the mass at 110° C.

The analysis of the product is shown in the accompanying table.

### *Antipeptone* (D).

The behavior of the preceding preparation while being dried, naturally suggested the suspicion that the substance was either decomposable at 100° C. or less in the air, or else that it contained some decomposable admixture. We therefore attempted a further purification of antipeptone and at the same time a more cautious method of drying.

For this purpose another preparation of antipeptone was made in the following manner.

230 grams of commercial dry pancreas, somewhat less active than that employed in the preceding preparation, were warmed at 40° C. for three hours with 1200 c. c. of 0·1 per cent. salicylic acid, after which the mixture was neutralized with sodium carbonate and to it was added directly 1920 grams of boiled, moist fibrin, 82 grams of dry sodium carbonate and 32 grams of thymol. This mixture was warmed at 40° C. for seven days, at the end of which time the residue of the pancreas, the antialbumid produced, and considerable separated tyrosin, formed a noticeable sediment, which was filtered off and pressed, after the residue had been thoroughly washed with water warmed at 40° C. The filtrate was made slightly acid, heated to boiling, and as this produced only a slight precipitate it was immediately concentrated to about three litres. On cooling, an abundant slate-colored precipitate separated, composed almost entirely of tyrosin. After removing this by filtration, the solution was saturated with ammonium sulphate and the resultant filtrate treated as in the

ANTIPEPTONE (C).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash. % | $BaSO_4$ from the ash. gram. | S from the ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5784 | 0·3323 | 6·38 | 0·9503 | 44·80 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·5949 | 0·3412 | 6·73 | 0·9776 | 44·81 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5771 | ---- | ---- | ---- | ---- | 74·36 | 5·0 | 763·0 | 15·95 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·4463 | ---- | ---- | ---- | ---- | 57·45 | 4·8 | 762·0 | 15·93 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 0·5256 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0278 | 5·28 | ---- | ---- | ---- | ---- | ---- |
| VI | 0·5092 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0270 | 5·32 | ---- | ---- | ---- | ---- | ---- |
| VII | 1·0348 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·17 |
| VIII | 0·5998 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0368 | 0·84 | 0·67 | 0·0130 | ---- |
| IX | 0·6149 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0390 | 0·87 | 0·70 | ---- | ---- |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 47·30 | 47·31 | ---- | ---- | ---- | ---- | *47·30* |
| H | 6·74 | 6·72 | ---- | ---- | ---- | ---- | *6·73* |
| N | ---- | ---- | 16·84 | 16·82 | ---- | ---- | *16·83* |
| S | ---- | ---- | ---- | ---- | 0·71 | 0·74 | *0·73* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *28·41* |
| | | | | | | | *100·00* |

previous case; that is, a barium peptone compound was formed, purified with alcohol and this exactly decomposed with sulphuric acid.

In order to separate the free peptone, the solution was concentrated at a gentle heat with the addition of a little ammonia, precipitated and boiled with alcohol, the almost liquid precipitate dissolved in water, the solution acidified with acetic acid, concentrated again, precipitated and extracted hot with alcohol, repeatedly washed and kneaded with ether, kept for a long time under ether and then slowly dissolved in as small an amount of cold water as possible. When filtered, a small residue of tyrosin appeared in the preparation. The new solution was concentrated at a gentle heat, precipitated again with alcohol, the peptone boiled and washed with alcohol, allowed to stand for some time with a large quantity of absolute alcohol, again treated with ether as before, and as it had now become friable, it was immediately dried in vacuo over sulphuric acid. On attempting to dry it at 100° C. the melting mass foamed so much that it had to be dried in the air. When this had been done with frequent stirring on the water bath for several days, the mass was finally dried completely *in vacuo*, first at 100° C., then at 105° C. A few weeks of this drying were needed to bring the substance to a constant weight, and in order to prepare the various quantities for analysis they had to remain *in vacuo* over sulphuric acid and at 105° C. for some time. Probably in consequence of the thorough treatment with ether, the preparation when warmed gave much less odor than the former one.

*Composition of Antipeptone* (D).

I. 0·5061 gram substance gave 0·2875 gram $H_2O$ = 6·31 per cent. H and 0·7937 gram $CO_2$ = 42·76 per cent. C.

II. 0·4449 gram substance gave 0·2527 gram $H_2O$ = 6·31 per cent. H and 0·7024 gram $CO_2$ = 43·05 per cent. C.

III. 0·5010 gram substance gave 0·2870 gram $H_2O$ = 6·36 per cent. H and 0·7885 gram $CO_2$ = 42·91 per cent. C.

IV. 0·4414 gram substance gave 56·0 c. c. N at 21·0° C. and 759·2 mm. pressure = 14·91 per cent. N.

V. 0·5870 gram substance gave 76·5 c. c. N at 21·6° C. and 758·0 mm. pressure = 15·13 per cent. N.

VI. 0·6930 gram substance gave 0·0694 gram ash = 10·01 per cent.

VII. 0·5017 gram substance gave 0·0504 gram ash = 10·04 per cent.

VIII. The ash from 0·5017 gram substance gave 0·0325 gram $BaSO_4$ = 0·89 per cent. S calculated on the original substance.

On account of the large percentage of ash and the large amount of sulphates, the sulphur of the organic matter was not determined.

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 47·52 | 47·83 | 47·69 | ---- | ---- | 47·68 |
| H | 7·01 | 7·01 | 7·07 | ---- | ---- | 7·03 |
| N | ---- | ---- | ---- | 16·57 | 16·80 | 16·68 |

*Antipeptone* (E).

In order to obtain a still purer preparation and especially to free it from the large percentage of ash, a portion of antipeptone (D) was dissolved in boiling water after the first treatment with ether, then when cold the solution was acidified with sulphuric acid to such an extent that it contained 6 per cent. of acid and precipitated with a large excess of phosphotungstic acid. The precipitate, after the manner already described under amphopeptone (B), was washed thoroughly with dilute sulphuric acid and then with water, finally decomposed with baryta, the barium-peptone precipitated with alcohol, washed, the alcohol driven off by heat, the aqueous solution of the compound exactly decomposed with sulphuric acid, the solution concentrated after the addition of a few drops of ammonia, precipitated with alcohol, dissolved again in water, concentrated with the addition of a little acetic acid, again precipitated with alcohol, and the product so obtained treated thoroughly with alcohol and ether in the same manner as preparation D, and finally dried in the same manner as that. The substance so prepared, was lighter colored than the preceding, not quite so hygroscopic, and in drying gave scarcely any odor.

The analysis of the product is shown in the accompanying table.

*Antipeptone* (F). (*Gland peptone.*)

This peptone was obtained as a bye product in a preparation of trypsin from 1,000 grams of dry pancreas and was formed wholly from the albuminous bodies of the gland substance, after extraction with alcohol and ether. It is not probable that the peptone contained, in any considerable quantity, any products from the digestion of elastin, since the elastic tissue could have been but little altered under the conditions in which the self-digestion of the gland took place during the preparation of the infusion, and furthermore there would have been needed for solution in the latter, a finer subdivision

ANTIPEPTONE (E).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. ° C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash. % | $BaSO_4$ from the ash. gram. | S from the ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·6561 | 0·3822 | 6·47 | 1·0840 | 45·05 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·6892 | 0·3989 | 6·43 | 1·1300 | 44·71 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·3911 | ---- | ---- | ---- | ---- | 59·68 | 22·8 | 756·7 | 17·61 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·7900 | ---- | ---- | ---- | ---- | 120·0 | 21·4 | 757·7 | 17·63 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 0·6379 | ---- | ---- | ---- | ---- | 96·63 | 21·4 | 757·6 | 17·58 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| VI | 0·5796 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0213 | 3·67 | ---- | ---- | ---- | ---- | ---- |
| VII | 0·6332 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0233 | 3·68 | ---- | ---- | ---- | ---- | ---- |
| VIII | 0·9190 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0500 | 0·74 | 0·63 | ---- | ---- |
| IX | 0·5438 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0300 | 0·76 | 0·65 | ---- | ---- |
| X | 1·2128 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0100 | 0·11 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 46·77 | 46·42 | ---- | ---- | ---- | ---- | ---- | *46·59* |
| H | 6·71 | 6·67 | ---- | ---- | ---- | ---- | ---- | *6·69* |
| N | ---- | ---- | 18·28 | 18·31 | 18·25 | ---- | ---- | *18·28* |
| S | ---- | ---- | ---- | ---- | ---- | 0·66 | 0·68 | *0·67* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *27·77* |
| | | | | | | | | *100·00* |

and long continued action. However this may be, the albumins of the pancreas naturally cannot be classified with the substances ordinarily used in digestion experiments, such for example as fibrin, without further investigation, for although there may be substances in the gland cells like serum-albumin, globulin and myosin, there are also many bodies quite different from these, as, for example, the leucoid precipitated by excess of acetic acid, zymogen and trypsinogen, all of which are decomposed by self-digestion and yield amido acids and peptones. So long as trypsin digestions are not ordinarily conducted with pure trypsin, it is of especial interest to find out the composition of the gland peptones, which, as a rule, have invariably been mixed in greater or less quantity with the antipeptones hitherto investigated.

*Preparation.*—1,000 grams of dry pancreas were warmed at 40° C. for twelve hours with five litres of 0·1 per cent. salicylic acid and 0·25 per cent. of thymol, filtered through muslin, the residue warmed another twelve hours with two litres of 0·25 per cent. sodium carbonate and 0·5 per cent. of thymol, again filtered and pressed, the two fluids united, brought up to an alkalinity of 0·25 per cent. of sodium carbonate and then warmed at 40° C. for three days.

After filtering through paper, the whole solution was slightly acidified with acetic acid and then saturated with five kilos. of ammonium sulphate, by which means there was precipitated a little albumose and all of the trypsin, the further treatment of which is of no interest here, while the gland peptone remained in solution. It is to be noticed in the separation of this peptone that it was treated exactly like preparation (C), excepting that the second purification with ether could be omitted. The preparation, after drying for some time, left a small residue of tyrosin when dissolved in cold water. It was therefore precipitated from this solution with alcohol, then freed from alcohol by boiling with water, dried directly over a water-bath and finally *in vacuo* at 106° C. until a constant weight was obtained.

The analysis of the product is seen in the following table.

*Antipeptone* (G). (Gland peptone.)

This preparation was obtained from the preceding product by the following process; the solution of the peptone was acidified with 6 per cent. of sulphuric acid, precipitated with a large excess of phosphotungstic acid, the precipitate carefully washed, then decomposed with barium hydroxide, the latter exactly removed with dilute sul-

ANTIPEPTONE (F) (*Glandpeptone*).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash. % | $BaSO_4$ from the ash. gram. | S from the ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·7511 | 0·4632 | 6·79 | 1·1652 | 41·97 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·3783 | 0·2301 | 6·75 | 0·5830 | 42·02 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5729 | ---- | ---- | ---- | ---- | 79·12 | 18·4 | 752·0 | 16·08 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·4792 | ---- | ---- | ---- | ---- | 66·36 | 18·6 | 754·0 | 16·15 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 0·6459 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | 0·0352 | 5·44 | ---- | ---- | ---- | ---- | ---- |
| VI | 0·6494 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | 0·0367 | 5·65 | ---- | ---- | ---- | ---- | ---- |
| VII | 1·2958 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0128 | 0·13 |
| VIII | 0·6509 | ---- | ---- | ---- | ---- | ---- | - | ---- | ---- | ---- | ---- | 0·0289 | 0·60 | 0·47 | ---- | ---- |
| IX | 0·6269 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | 0·0270 | 0·59 | 0·46 | ---- | ---- |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 44·42 | 44·48 | ---- | ---- | ---- | ---- | *44·45* |
| H | 7·19 | 7·15 | ---- | ---- | ---- | ---- | *7·17* |
| N | ---- | ---- | 17·02 | 17·09 | --- | ---- | *17·06* |
| S | --- | ---- | ---- | ---- | 0·51 | 0·50 | *0·50* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *30·82* |
| | | | | | | | *100·00* |

phuric acid, the solution concentrated, the peptone precipitated and washed with alcohol and finally boiled with alcohol. The partially dry product was then dissolved in cold water, leaving a small amorphous residue which gave no reaction for tyrosin. The solution was then concentrated on the water-bath and again precipitated with alcohol, after which it was dried finally, over sulphuric acid in vacuo and at 106° C. in vacuo, until of constant weight.

The following table shows the results of the analysis.

*Antipeptone* (H). (Gland peptone.)

This product was prepared and purified in exactly the same manner as the former one (G), but was made from another trypsin preparation, in which a smaller amount of dry pancreas was used. When dissolved for the last time in cold water, some little insoluble matter remained (from which it was freed) which, however gave no reaction for tyrosin.

The following table shows the results of the analysis.

GENERAL PROPERTIES OF THE PEPTONES.

We should have liked to study more accurately the physical behavior of the different samples of peptones, especially their optical properties as determined by specific rotary power. It was easy to show that they were all laevo-rotary, but we have not yet succeeded in making any quantitative determinations of sufficient accuracy to be of value. The decidedly brown color of the solutions prevented the use of a sufficiently long tube, or a solution of the proper concentration, necessary to determine specific rotation.

It would be of still greater importance to investigate the rate of diffusion of peptones, it being more necessary from the fact, that previous observations on the diffusion of the products of digestion can have but little reference to pure peptones, but rather to the albumose bodies so long overlooked. We have, however, not as yet begun these investigations owing to lack of material. All of the peptones obtained by us in the dry state, showed considerable rise of temperature when moistened with, or dissolved in water.

It is worthy of notice, physiologically, that according to observations made in the physiological Institute at Heidelberg, by Dr. Pollitzer of New York, no one of the peptones would hasten or retard coagulation of the blood, either when injected into the veins or added to the shed blood, such action being due wholly to certain of the albumose bodies.

ANTIPEPTONE (G) (*Glandpeptone*).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ found. gram. | S % | S after deducting S of ash. % | $BaSO_4$ from the ash. gram. | S from the ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4914 | 0·3163 | 7·15 | 0·7600 | 42·17 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·6475 | 0·4141 | 7·10 | 0·9998 | 42·10 | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·6381 | ---- | ---- | ---- | ---- | 94·8 | 17·6 | 762·2 | 17·58 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·4756 | ---- | ---- | ---- | ---- | 69·6 | 17·4 | 763·8 | 17·37 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 0·6898 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | 0·0132 | 1·91 | ---- | ---- | ---- | ---- | ---- |
| VI | 0·6732 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | 0·0132 | 1·96 | ---- | ---- | ---- | ---- | ---- |
| VII | 1·3630 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0087 | 0·09 |
| VIII | 0·8856 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | 0·0249 | 0·88 | 0·29 | ---- | ---- |
| IX | 0·6059 | ---- | ---- | ---- | ---- | ---- | -- | ---- | ---- | ---- | ---- | 0·0178 | 0·40 | 0·81 | ---- | ---- |

***Percentage composition of the ash-free substance.***

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 42·99 | 42·93 | ---- | ---- | ---- | ---- | *42·96* |
| H | 7·29 | 7·24 | ---- | ---- | ---- | ---- | *7·26* |
| N | ---- | ---- | 17·70 | 17·91 | ---- | ---- | *17·80* |
| S | ---- | ---- | ---- | ---- | 0·80 | 0·82 | *0·31* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *31·67* |
| | | | | | | | *100·00* |

ANTIPEPTONE (H) (*Glandpeptone*).

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. ° C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ found. gram. | S % | S after deducting S of ash. % | $BaSO_4$ from the ash. gram. | S from the ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4107 | 0·2607 | 7·05 | 0·6548 | 43·47 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·5174 | 0·3243 | 6·96 | 0·8280 | 43·64 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5940 | ---- | ---- | ---- | ---- | 88·48 | 18·6 | 762·8 | 17·58 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·5117 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0106 | 2·07 | ---- | ---- | ---- | ---- | ---- |
| IVa | 0·5117 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0088 | 0·10 |
| V | 0·4969 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0240 | 0·66 | 0·56 | ---- | ---- |

*Percentage composition of the ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 44·39 | 44·56 | ---- | ---- | *44·47* |
| H | 7·20 | 7·11 | ---- | ---- | *7·15* |
| N | ---- | ---- | 17·94 | ---- | *17·94* |
| S | ---- | ---- | ---- | 0·57 | *0·57* |
| O | ---- | ---- | ---- | ---- | *29·87* |
| | | | | | *100·00* |

A few observations on the taste of peptones are of interest. While the genuine albumins and the albumose bodies excite practically no sense of taste, the less so the purer they are, it appears as if peptones belong to the most offensively tasting bodies. In order to see what an important change the taste of an albuminous body undergoes on digestion, and at the same time what the taste of peptones is, warm 50 c. c. of fresh milk to 40° C. and then add to it a small fragment of soluble trypsin (prepared from ox pancreas), which excites no taste of itself. The milk at first coagulates, then regains its former appearance by solution of the coagulum, but tastes no better than gall. Nevertheless we believe that the especially objectionable taste proceeds not from peptones, but from certain compounds heretofore only accidentally separated from them. For, among our preparations, which as a whole tasted something like roast meat, as if burnt, but above all nauseatingly bitter and astringent even in a 2 per cent. solution, we found one that in a 10 per cent. solution was free from this disagreeable peculiarity and had a pleasant, sweet taste like meat. It is to be noticed that this was the preparation of antipeptone (F) which had not been purified by phosphotungstic acid. Only by taking a large swallow was there noticed a not unpleasant taste, peculiar to certain cheeses after becoming alkaline.

The most important property for the separation and treatment of peptones is for the present, the lack of precipitation by a great number of reagents, which precipitate more or less perfectly albumins and albumose bodies, especially sodium chloride, either alone, or with the addition of acid. We have repeatedly confirmed the observations made by Wenz that even sodium chloride and acetic acid, sodium chloride and nitric acid or metaphosphoric acid do not completely precipitate the albumose bodies. In whatever proportion these additions may be made, there always remains at the end a solution which gives with alcohol a precipitate of salt, in which albumose is still to be found, or from which albumose may be separated by dialysing and concentrating, according to the method already used. The only perfect precipitant of these substances is ammonium sulphate. It is however an error to attribute to this salt the same action on peptones. Wherever peptones occur, they will always be found in the filtrate from a solution saturated with ammonium sulphate, and we must conclude from some opposed statements that in the experiments on which they are based, albumose bodies instead of peptones were present, since we are certain that by means of our new method, it can be generally shown what an unexpected difference exists between the ap-

parently vigorous action of a poorly prepared gastric juice, or commercial preparation of pepsin and the action of solutions actually rich in pepsin. Even where the fibrin almost instantly disappears, the amount of ferment may still be quite insufficient to produce noticeable traces of peptones. Therefore care must be taken not to conclude immediately from a speedy solution, that digestion has been complete, since this is to be determined only by the disappearance of the primary cleavage products of digestion, that is, the change of albumose into peptones.

It was especially interesting to ascertain whether peptones isolated according to the methods already described, were likewise precipitated by ammonium sulphate or by other reagents that precipitate the albumose bodies, a question which was interesting considering the oft-asserted formation of albumin or albumose from peptones. To our surprise we noticed in the beginning that both antipeptone and amphopeptone after complete purification, under certain circumstances gave rise to a turbidity or even a resinous precipitate, not only with ammonium sulphate, but also when their solutions were saturated with salt or when treated with acetic acid, nitric acid or metaphosphoric acid, just as if albumose had been formed or the albumose not completely removed by the previous treatment. Even if these precipitates concerned only a small part of the material in solution, their appearance would need explanation. So far as we can now determine, the occasion of this behavior is a circumstance concerning which we do not care to decide whether it really depends on the formation of albumose from peptone or not. It is to be observed that if the purification of the peptone by sulphuric acid is conducted incautiously, either on decomposing the barium-peptone or on acidifying before precipitation with phosphotungstic acid, the appearance afterward of albumose is avoided provided the solutions, when warm, are never exposed to an excess of acid. That a resinous precipitate appears, while boiling the solution saturated with ammonium sulphate at 110° C. has already been mentioned, but this cannot cause any impurity of the peptone remaining in solution, any more than the well known precipitation of antialbumid during trypsin digestion can occasion an impurity of the antipeptone.

Since the fact is proved that peptones are not precipitated by ammonium sulphate, these bodies are then characterized more than ever by the property long attributed to them of being rendered turbid by very few reagents and completely precipitated by a still more limited number. A list of the latter reagents includes only tannin

and mercuric iodide in potassium iodide, while imperfect precipitants of peptones are phosphotungstic acid or phosphomolybdic acid and picric acid.

The following list shows the further action of various reagents.

*Reactions of Peptones free from albumose and purified by phosphotungstic acid.*

In 5 per cent. solution, after being made noticeably alkaline with a trace of sodium carbonate.

| | Fibrin antipeptone. | Fibrin amphopeptone. |
|---|---|---|
| Acetic acid and potassium ferrocyanide. | At first perfectly clear, later trace of opalescence. | The same. |
| Neutral lead acetate. | First drop, 0; more, turbidity. | The same, but much weaker. |
| Basic lead acetate. | Turbidity immediately; more, strong turbidity. | The same, but weaker. |
| Mercuric chloride. | First drop, 0; more, strong turbidity. | Turbidity immediately, growing stronger. |
| 5 per cent. cupric sulphate. | At first clear; more, slight turbidity disappearing with great excess. | Nothing. |
| 5 per cent. platinum chloride. | Only excess, strong turbidity. | Nothing. |
| Chromic acid. | Nothing. | Nothing. |
| Ferric chloride. | A trace gives turbidity vanishing with the least excess. | Nothing. |
| Glacial acetic acid and conc. sulphuric acid. | Brownish red. | The same. |
| Nitric acid. | The color changing yellow in the cold. | The same. |
| Boiling with conc. hydrochloric acid. | The color becomes slightly darker. | The same. |
| Millon's reaction. | At first a heavy white precipitate; on warming, dirty yellow or reddish. | The same, then beautiful red color. |

Without desiring to claim especial value in general for these reactions and for smaller differences between the two peptones, some of them, however, may be more closely examined.

The slight intensification of color by boiling with concentrated hydrochloric acid is striking, for we have not ordinarily been able to obtain it, even with peptone in substance or even on the addition of

concentrated sulphuric acid. Likewise, the reaction with sulphuric acid and glacial acetic must be described as almost unsuccessful. As this, however, is nothing other than Pettenkofer's test for bile acids, for which its discoverer has recommended concentrated acetic acid as a substitute for sugar, we have also tried the reaction with sugar, without however obtaining any better result, particularly not the beautiful violet-red which albumin and the albumose bodies give. Finally, the poor result of Millon's reaction with antipeptone, in contrast to the brilliant red obtained with amphopeptone, is also to be remarked. To this reaction we shall return later.

It has already been observed by many investigators that among the products of the digestion of albumin, bodies are not infrequently met with, which give little or no lead sulphide on boiling with sodium hydroxide and lead acetate. This is not at all strange, since the peptones prepared by us show on analysis less than one per cent. of sulphur, in striking contrast to the albumins and albumose bodies, all of which contain much larger percentages. In the gland peptones (F, G, H,) the sulphur amounted to only 0·50, 0·31 and 0·57 per cent. respectively. The peculiarity of peptones in giving up a part of their sulphur when warmed with alkali, apparently stands in no direct connection with the percentage amount of sulphur. Indeed, solutions of the antipeptones G and H, of which the first possessed the lowest percentage of sulphur found, showed no browning with this test, and only a trace of it when solid particles of the peptone were heated with a concentrated solution of alkali containing lead. But the gland peptone (F) not purified by phosphotungstic acid and with only 0·15 per cent. of sulphur became slightly darkened in solution. On the other hand, the purest amphopeptone (B) with 0·77 per cent. of sulphur gave the reaction very faintly, while antipeptone C with 0·73 per cent. of sulphur gave it very plainly. Probably the reaction is not to be attributed to the peptones themselves, but proceeds from contamination with an easily decomposed substance containing sulphur, whose removal still depends on chance.

It may be assumed as completely proved that the rose or violet coloration, which the products of pancreatic digestion assume with bromine or chlorine water, is due to some special body and not to antipeptone. We had previously shown this to be the case with the antipeptone obtained by the action of trypsin from antialbumid, and have now also found in all antipeptones purified with phosphotungstic acid, the absence of all color on addition of bromine water, either in large or small quantity.

Finally, not to overlook the most striking reaction of peptones, we mention further the brilliant so-called biuret reaction which is to be seen, if possible, still more intense in the purer peptones, whatever their origin, than in those formerly used.

### *Composition of the Peptones.*

For the sake of comparison we present in the following table the percentage composition of the various samples of peptones, calculated on the ash-free substance. The ash found, consisting in every case of calcium, a little sodium, potassium, traces of barium and iron, carbonic acid, phosphoric acid and sulphuric acid, is placed at the foot of the columns.

| | Ampho- (pepsin) peptone from fibrin. | | | Anti- (trypsin) peptone. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A. | B. | b. | C. | D. | E. | F. | G. | H. |
| | | | | Prepared from fibrin. | | | Glandpeptone. | | |
| | Containing mucin-peptone. | From purified pepsin and purified with phosphotungstic acid. | | | Purified more with ether. | Purified with phosphotungstic acid. | | Purified with phosphotungstic acid. | |
| C | 44·53 | 48·75 | 48·47 | 47·30 | 47·68 | 46·59 | 44·45 | 42·96 | 44·47 |
| H | 6·49 | 7·21 | 7·02 | 6·73 | 7·03 | 6·69 | 7·17 | 7·26 | 7·15 |
| N | 16·73 | 16·26 | 16·86 | 16·83 | 16·68 | 18·28 | 17·06 | 17·80 | 17·94 |
| S | 0·72 | 0·77 | .... | 0·73 | .... | 0·67 | 0·50 | 0·31 | 0·57 |
| O | 31·53 | 27·01 | .... | 28·41 | .... | 27·77 | 30·82 | 31·67 | 29·87 |
| Ash | 8·11 | 3·22 | 2·15 | 5·25 | 10·02 | 3·67 | 5·54 | 1·93 | 2·07 |

The nature of the substance, which at present affords but little proof that we have to deal with a simple body and not a mixture of chemical bodies, places the greatest restrictions on the use of the above values, and only under such reserve is to be understood what is hereafter added. As regards the analysis of the single substances, we are inclined to believe that only in amphopeptone B, is there a difference in the percentage of carbon greater than the ordinary differences naturally to be expected in amorphous materials so difficult to prepare.

Amphopeptone A, with the lowest percentage of carbon, has already been described as a mixture of fibrin-peptone and mucin-peptone. If we have succeeded in removing the latter, by using

purified pepsin, then the figures obtained from *B* and *b* may be considered as representing the first gastric peptone prepared free from albumose. The correspondence between the two, in spite of the fact that by continued purification of *b* we succeeded in reducing the ash by more than 1 per cent., we think may be considered as grounds for this assumption. In opposition therefore to the majority of previous statements, including our own, which as now easily understood referred to mixtures of albumose and peptones, there is to be noticed in pure amphopeptone about 1 per cent. lower content of carbon, about as much higher a percentage of nitrogen and 0·3–0·4 per cent. lower content of sulphur.

With antipeptone, the variation from the previous results was less expected, for we are not inclined to believe that in our former long and thorough typsin digestions, any appreciable quantity of albumose remained and in the more recent ones, the precipitate produced by ammonium sulphate was never abundant if the matter separated by boiling in a slightly acid solution was previously removed. The differences found, however, might be readily explained by the fact that the purification of the peptone had this time been more complete, owing partly doubtless to the formation of the barium compound, and partly also to the precipitation with phosphotungstic acid. The content of carbon is seen to be about 1 per cent. lower, the content of sulphur likewise lower and the percentage of nitrogen decidedly higher, in one case as much as 4 per cent. more than before. The real reason for this difference in composition appears to us to lie in the use of much larger quantities of trypsin, which formerly was only possible by using large quantities of the gland substance, so that the fibrin-antipeptone would naturally be obtained mixed with the gland peptone. It is well for the future that we know the composition of these gland peptones, for they differ essentially, in the lower content of carbon (in one case 42·96 per cent.), from all other peptones hitherto investigated. These bodies might be pronounced troublesome intruders with the same right as the mucin-peptone arising from gastric digestion, although we found in it, aside from the percentage composition, no reaction and no property which would serve to distinguish it from the other peptones.

After these considerations, little stress can be laid on the differences between the composition of pepsin and trypsin peptones. We have found, however, another difference which we will examine more closely.

*Cleavage of the peptones.*

As already mentioned, Millon's reaction appears very brilliant with amphopeptone, but more or less imperfectly with antipeptone. As the reaction is sure only under certain circumstances we have not neglected to perform it in every possible way, either by using the same concentration of solutions of the two peptones under exactly the same conditions, or by trying the most successful variations of the experiment for antipeptone. Thus it was found that the preparations C, D, E, and F, treated in a suitable way, gave an appreciable reaction, but in no case so that more than a dirty, generally orange red precipitate was obtained. On the contrary, all trials with gland peptone purified with phosphotungstic acid, failed to give more than a simple yellow color. Since Millon's reaction for albumin corresponds with the so-called Hoffmann's test for tyrosin, and since with albumin it probably depends on the separation of tyrosin by boiling with the acid solution of murcuric nitrate, if not by the formation of further decomposition products of tyrosin (hydroparacumaric acids) which likewise redden with the test, it might be presumed that antipeptone, in contrast to the amphopeptone of pepsin digestion, forms no tyrosin by cleavage. So far as the action of trypsin is concerned, this was already known, since the real difference between gastric and pancreatic peptones consists in the fact that only the former, when treated with trypsin, yield tyrosin together with leucin and other decomposition products—in our opinion because they contain hemipeptone capable of further cleavage (together with antipeptone). It was also known to us, however, that antipeptone during cleavage with boiling sulphuric acid yields the amido acids, and among them also tyrosin. Renewed investigations on this subject appeared called for now, since we thought ourselves in possession of much purer preparations of antipeptone.

First, we established the possibility of decomposing with trypsin, amphopeptone entirely free from albumose. A few hours' digestion in a small test tube, of 1 gram of peptone in 10 c. c. of water, containing 0·25 per cent. of sodium carbonate with a little thymol and a fragment of purified pepsin, sufficed for this purpose. By concentrating the neutralized solution and boiling the residue with alcohol, a decided residue was obtained in which balls of leucin and bundles of tyrosin were to be seen under the microscope without further preparation. The residue was also colored a beautiful violet with bromine water. We also sacrificed a large quantity of amphopeptone to the same experiment and obtained the tyrosin pure (free

from peptone) and tested it, both by Millon's reaction and Piria's test. Further, a few grams of the same preparation were heated for several days with six times its weight of sulphuric acid (2 : 3 water), and after removing the acid with barium hydroxide, tyrosin and leucin were found among the decomposition products.

Antipeptone was now likewise submitted to the above treatment. The method of conducting this experiment with albuminous bodies which we have used successfully, even with 2–3 grams of substance, is as follows. The substance is placed in a small, strong flask standing on an asbestos plate, and five or six times the weight of sulphuric acid of the above mentioned strength is added and the mixture kept boiling as long as desired. The loss of water by evaporation is prevented by connecting to the neck of the flask a glass tube 1 metre long and 1 centimetre in diameter, so that the lower end cannot be closed by a drop of water. The upper end of the tube is drawn out to a capillary point and as it does not become warm at the top during the boiling of the fluid, all loss of water is prevented.

To be as sure as possible, we have treated the antipeptone in this manner for 48 hours. The contents of the flask were then much diluted with water, filtered from the sticky residue always present, made alkaline with a concentrated solution of barium hydroxide, the barium separated from the filtrate by sulphuric acid, after which the clear fluid was concentrated and allowed to crystallize. It is not advisable to remove the sulphuric acid with barium carbonate, since the latter is unavoidably used in excess, for it is very undesirable to have the barium precipitate unnecessarily increased in bulk, as the cleavage products of albumin are difficult to remove even with hot water. Further, the mixure is made alkaline by barium carbonate and soluble barium compounds always appear, which must be removed by sulphuric acid in every case.

In the strongly concentrated solution finally obtained, in case leucin and tyrosin have really been formed, as with most albuminous bodies, their presence may be readily shown microscopically and after suitable separation of the mother liquor, or if necessary, after re-crystallization from dilute alcohol, their chemical reactions may likewise be obtained. We have succeeded after this manner in showing the presence of leucin always in antipeptone; tyrosin, however, only in a few cases, and even then only after repeated crystallization. Whenever tyrosin occurred it was in exceedingly small quantities. From the antipeptones (gland peptones H and G) with which Millon's test had hitherto failed, tyrosin could not be obtained at all, and that

tyrosin was really absent in this case was shown by the final examination of the whole united residues by Hoffman's test, which was absolutely negative, while it appeared plainly with the products of the remaining antipeptones.

Although we do not wish to consider the behavior of the gland peptones as a criterion for antipeptone in general, still this result, united with the small amount of tyrosin obtained from the latter, seemed to call for further investigations concerning the decomposition of those primary cleavage products of albumin related to the antipeptones, especially antialbumid. We began this extension of our work because during our treatment of the subject an article of Maly's* appeared, in which he described a very interesting cleavage and oxidation product, obtained by treating albumin with potassium permanganate, which product possesses the essential properties of the albumins, and yet on further decomposition does not yield tyrosin. It is questionable, therefore, whether this property is not the one directly distinguishing the bodies of the anti-group from the primary cleavage products of the albumins.

A few preliminary experiments were made with samples of antialbumid prepared by the action of boiling dilute sulphuric acid, both on fibrin and Thiry's neutralization precipitate from egg-albumin, also with the antialbumid remaining from the digestion of fibrin with trypsin, and finally with a small neutralization precipitate of so-called parapeptone from an incomplete pepsin digestion of fibrin, which we regarded as antialbumose. After these experiments as a whole, had resulted contrary to our expectations, in that a moderate amount of tyrosin appeared after boiling the substance for a long time with sulphuric acid, we submitted to decomposition a preparation from which we thought we could expect a decisive result. This preparation was an antialbumid from egg albumin, made in one of our former investigations as follows: White of egg freed from membrane, was coagulated by heat in an acid solution, the coagulum filtered, thoroughly washed and then heated for a long time at 100° C. with dilute sulphuric acid, the residue filtered, washed thoroughly with water, dissolved in sodium carbonate, precipitated by neutralization, the precipitate dissolved in 0·2 per cent. hydrochloric acid and the antialbumid freed from all other other albuminous bodies by long continued digestion with pepsin. The antialbumid was then separated from the solution by neutralization, washed, dissolved in 0·5 per

* Wiener Acad. Sitzungsber., xci, Abth. 5, February, 1885.

cent. sodium carbonate, warmed with trypsin at 40° C., and after the antialbumid had partially separated as a jelly-like mass, it was filtered and washed with water, finally with alcohol and ether. This product could not possibly contain any known albumin, albumose or peptone and undoubtedly formed the purest sample of antialbumid yet prepared.

By decomposing this body with sulphuric acid, a residue was finally obtained which to our surprise gave but the slightest reaction for tyrosin with Millon's and Hoffmann's test, with Piria's test no reaction whatever, and in spite of endeavors continued for weeks not a single crystal of tyrosin could be detected. Leucin was found in very small quantity and in addition there were seen large lustrous balls of crystals of some nitrogenous substance, too small in quantity to be identified.

Heidelberg, } December, 1885.
New Haven, }

## XVI.—On the dehydration of Glucose in the Stomach and Intestines. By R. H. Chittenden.

In a series of interesting communications* "On the physiology of the carbohydrates in the animal system," Dr. F. W. Pavy has brought forward evidence to show that glucose, generally considered as the final product of amylolytic action, can be converted within the animal body into a product of less cupric oxide-reducing power; that there exists particularly in the stomach and intestines of rabbits, a ferment which has a dehydrating action upon glucose or dextrose, transforming it into a body akin to maltose in reducing power.

Hitherto, it has been generally supposed that the transformations which carbohydrates undergo in the animal system are in the nature of gradual hydration changes, in which each step forward toward the final product is attended with the formation of bodies of increased cupric oxide-reducing power. Dr. Pavy's results, however, would tend to show that transformations in the opposite direction do occur and this notably in the stomach and intestines of rabbits.

Dr. Pavy's conclusions concerning this dehydration of glucose in the animal system, are based upon changes in the cupric oxide-reducing power of the carbohydrate, after contact with portions of the stomach and intestines for short periods of time at 48·8° C. It is a well known fact that the reducing power of pure glucose is not affected by boiling with dilute sulphuric acid, while under like treatment, maltose and similar bodies are readily converted into glucose or into a body of like cupric oxide-reducing power.

Dr. Pavy finds, as the result of a large number of experiments, that a solution of glucose or grape sugar, by mere contact with the stomach and intestines of a rabbit at 48·8° C. is changed into a body of less cupric-oxide reducing power, and that by boiling with dilute sulphuric acid this product is carried back again into glucose. Thus, in one experiment, 0·138 gram of glucose in contact with strips of stomach from a rabbit for one hour and a half at 48·8° C. showed, after removal of the dissolved albumin by boiling with sodium sulphate, a reducing power calculated to the entire amount equivalent to only 0·080 gram of glucose; while after boiling with dilute sul-

* Chemical News, 1884, vol. xlix, pages 128, 140, 155, 162, 172 and 183.

phuric acid (the solution containing two per cent. $H_2SO_4$) the cupric oxide-reducing power was increased to the equivalent of 0·134 gram of glucose, or nearly equal to the amount started with.

It is to be noticed in the experiment just quoted, that the cupric oxide-reducing power, before and after treatment with sulphuric acid, stand to each other in the proportion of 58 : 100, or in about the relation of maltose (61) to glucose (100). In some experiments, however, the reducing power before boiling with dilute acid, was so low as to warrant the belief that dextrins were also formed. This result is a type of many similar ones obtained by Pavy with the stomach and intestines from various animals and in no instance, in the case of rabbits at least, so far as reported, were negative results obtained.

The discovery of such a dehydrating ferment, hitherto unsuspected, appeared to be a matter of so much importance that experiments have been tried in this laboratory from time to time during the past two years, with the view of confirming in part at least some of Dr. Pavy's results. To our surprise, however, in no case, have we been able to obtain results corresponding to those of Pavy's, although the animals experimented with (rabbits and cats) were taken in various stages of digestion. We therefore record here, some of the results simply in the hope that some light may be thrown upon the cause of this discrepancy; or if, as may be, the ferment is not invariably present, some reason may be found for its constant absence in the tissues of the animals experimented with, and thus light be thrown upon the conditions which control its presence.

The glucose used in the following experiments was a sample of crystallized anhydrous glucose presented to the laboratory by Dr. Arno Behr. The sugar was quite pure, as was ascertained by testing both its reducing power and specific rotary power, and more important still, was not at all affected by boiling with dilute sulphuric acid. Thus 50 c. c. of a one per cent. solution of the glucose, mixed with sufficient 10 per cent. sulphuric acid to have the mixture contain two per cent. of $H_2SO_4$, was boiled for one and one-half hours, the flask being connected with an inverted Liebig's condenser to prevent concentration. The solution was then neutralized, diluted to 100 c. c. and tested with Fehling's solution according to the method of Allihn. 25 c. c. yielded 0·2414 gram of metallic copper, corresponding to 0·1246 gram of glucose, whereas the 25 c. c. of solution should have contained 0·1250 gram of sugar. Evidently then, the reducing power of the sugar is not affected by treatment with dilute acid.

*First experiment.*—A rabbit in full digestion was killed, the stom-

ach emptied of its contents and then divided into two longitudinal halves along the curvatures. One-half, after being cleansed, was finely divided and placed in a small beaker with 70 c. c. of water containing 0·200 gram of glucose. An equivalent amount of the small intestine, similarly cleaned and divided, was placed in a second beaker in contact with 70 c. c. of water containing 0·150 gram of glucose. In both cases the entire walls, including *muscularis* and *mucosa*, were taken, since Pavy has indicated that the converting principle is situated not on the surface of the mucous membrane, but in the deeper part. The two mixtures were then placed in a bath and warmed at 48·8° C. for nearly two hours; after which they were boiled, crystals of sodium sulphate being added to aid the removal of the dissolved albumin. The individual filtrates and washings were concentrated and finally brought to a volume of 100 c. c. Of this, 25 c. c. were used to determine the cupric oxide-reducing power of the solution directly, while 50 c. c. of each solution were mixed with sufficient ten per cent. sulphuric acid to insure a content of two per cent. and then boiled for two hours, in connection with an inverted Liebig's condenser to prevent concentration. The acid solutions were then neutralized, concentrated somewhat and finally brought back to a volume of 50 c. c. Following are the analytical results obtained with the two solutions, the reducing power being determined by Allihn's gravimetric method.*

STOMACH.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0746 gram Cu=0·0381 gram dextrose ×4=0·1524 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0728 gram Cu=0·0372 gram dextrose ×4=0·1488 gram dextrose.

INTESTINE.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0512 gram Cu=0·0265 gram dextrose ×4=0·1060 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0520 gram Cu=0·0269 gram dextrose ×4=0·1076 gram dextrose.

Here, there is no evidence whatever that the glucose was affected by its two hours' contact with the stomach and intestine of the rabbit at 48·8° C., the temperature specified by Pavy as that best adapted for the reaction. Certainly the reducing power of the glucose solution is essentially the same before and after treatment with sulphuric

* Zeitschrift für Analytische Chemie, 22. Jahrgang, p. 448.

acid. Somewhat in accord with Pavy's results, however, is the fact that while 200 milligrams of glucose were introduced into the stomach mixture and 150 milligrams with the intestines, only 152·4 milligrams were recovered from the former and 106 milligrams from the latter, although the residues after heating with sodium sulphate, were repeatedly and thoroughly washed with hot water. Assuming that this loss of sugar in the two cases is really due to change of glucose into lower reducing bodies, the relative reducing power of the sugar before and after contact with the stomach and intestines would be 100 : 76·2 and 100 : 70·6 respectively. But if there had been any such change in reducing power, the treatment with sulphuric acid would certainly have indicated it.

*Second experiment.*—A rabbit in full digestion was killed and half of the stomach and a portion of the small intestine were cleaned and finely divided. The stomach tissue was then heated at 48·8° C. for one and one-half hours, with 70 c. c. of water containing 0·200 gram of glucose and the portion of intestine for the same length of time, with a like amount of glucose. Treated then in the same manner as the preceding solutions, the following results were obtained:

STOMACH.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0901 gram Cu =0·0460 gram dextrose ×4=0·1840 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0882 gram Cu =0·0450 gram dextrose ×4=0·1800 gram dextrose.

INTESTINE.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0690 gram Cu =0·0353 gram dextrose ×4=0·1412 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0726 gram Cu =0·0371 gram dextrose ×4=0·1484 gram dextrose.

In this experiment, a larger amount of sugar was recovered in the case of the stomach tissue than in the preceding experiment, but in neither the stomach or intestine is there any evidence of change in the reducing power of the sugar before and after treatment with sulphuric acid. In this connection it is to be remembered, that the only ground for belief in the existence of a dehydrating ferment in the stomach is the change, noticed by Pavy, in the reducing power of the sugar under the above method of treatment. Our method of treatment with sulphuric acid, moreover, both as to the length of time the mixtures were heated and the strength of acid employed, was in accord with the method used by Pavy. In addition, the same

method of treatment was applied to a known solution of maltose with satisfactory results, viz: a rapid and complete change into dextrose as attested by the proper proportional increase in reducing power. Furthermore, we are led to infer from Pavy's results that the action of the ferment is to be seen to the best advantage in the rabbit. Thus Pavy states,* that without having made any precise comparative observations, 'I am under the impression that the stomach and the intestine of the rabbit act more energetically than the stomach and intestine of the other animals I have tried. It also appears to me that the stomach acts more energetically than the intestine, and in some instances I have noticed that the effect produced, has stood in relation to the amount of ferment material used." The latter half of this statement would tend to indicate that the main reason for our not recovering all of the glucose is to be found either in a lack of sufficient washing of the tissue residue at the end of the experiment, or else in a slight fermentation by which a portion of the sugar might be decomposed; for as is to be noticed in nearly all of the experiments recorded here, far less sugar is lost in the stomach than in the intestine, whereas if due to change in reducing power from the action of a dehydrating ferment, the greatest loss, Pavy's statement being correct, would be observed by contact with the stomach tissue. On the contrary, our results show greatest loss in the intestine, which if due to mechanical reasons would be naturally explained, since the glairy mass of tissue, even after boiling, affords mechanical obstacles to a thorough extraction. That this is doubtless the true explanation, in part at least, is evidenced by the fact that a portion of the stomach or intestine, previously boiled with water to destroy its vitality, yields results after the same order as those already given, except that the amount of sugar recovered is greater, as would naturally be expected since the tissue being already coagulated would not enclose the sugar so completely. Thus, on warming one-half of a rabbits' stomach, previously divided and boiled with water, with 0·200 gram of glucose for two hours, there was recovered 0·1910 gram of the glucose; while from a portion of the small intestine, likewise boiled and treated with the same amount of glucose, there was recovered only 0·1840 gram of the sugar. Furthermore, fermentation of the sugar would naturally occur more quickly in the intestines than in the more compact stomach tissue. Be that as it may, the reducing power of neither solution was affected by boiling with dilute sulphuric acid.

* Chemical News, vol. xlix, p. 141.

*Third experiment.*—A rabbit in a condition of hunger was killed, the stomach divided longitudinally along the curvatures, and one-half after being cleaned and finely divided, was placed in contact with 0·200 gram of glucose dissolved in 70 c. c. of water, and warmed at 48·8° C. for one and one-half hours. The mixture was then heated to boiling with the addition of some crystals of sodium sulphate, the tissue and coagulated albumin filtered off and the residue washed with about 300 c. c. of hot water. The fluid was concentrated, brought to a volume of 100 c. c. and then treated as in the preceding experiments. Following are the results obtained:

STOMACH.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0834 gram Cu = 0·0425 gram dextrose × 4 = 0·1700 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0798 gram Cu = 0·0407 gram dextrose × 4 = 0·1628 gram dextrose.

Here, as before, there is no evidence of any change in the character of the glucose; still in spite of the comparatively large volume of wash-fluid used, the sugar was not wholly recovered.

*Fourth experiment.*—A cat killed in full digestion was employed in this experiment. One-half of the stomach, finely divided, was placed in contact with 0·200 gram of glucose in 75 c. c. of water. A portion of the small intestine was also treated with a like amount of sugar, in the same manner. Both were warmed for three hours at 48·8° C., then treated by the same method as used in the preceding experiments.

STOMACH.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0879 gram Cu = 0·0449 gram dextrose × 4 = 0·1796 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0886 gram Cu = 0·0452 gram dextrose × 4 = 0·1808 gram dextrose.

INTESTINE.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0668 gram Cu = 0·0342 gram dextrose × 4 = 0·1368 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0684 gram Cu = 0·0350 gram dextrose × 4 = 0·1400 gram dextrose.

Here again, there is no evidence whatever of any change in the reducing power of the sugar solution.

Pavy has also pointed out that the stomach and intestine of the rabbit, as well as of other animals, have a transformative action on saccharose as well as on dextrose. The transformative energy how-

ever of the intestine, is much greater in this case than that of the stomach; which, according to Pavy, accounts for the early discovery by Bernard of the well known action of the intestine on cane sugar. Bernard supposed dextrose to be formed, but Pavy shows that maltose or a body resembling maltose in reducing properties, is the usual product and that glucose or dextrose is formed only in the presence of considerable ferment. The ferment which produces this change, unlike the ferment which acts upon glucose, is situated on the surface of the mucous membrane and thus frequently the contents of the stomach are likewise found to possess transformative power.

*Fifth experiment.*—A rabbit with stomach partially filled with food was killed, the stomach rinsed with water, then minutely divided and separated into two equal parts. One portion was placed in contact with 0·195 gram of glucose in 75 c. c. of water, while the other portion was mixed with a like amount of pure saccharose, also in 75 c. c. of water. A portion of the small intestine was likewise finely divided and one portion placed in contact with 0·195 gram of glucose in 75 c. c. of water and the other portion with 0·200 gram of saccharose dissolved in 75 c. c. of water. All four mixtures were warmed at 48·8° C. for two hours, then heated to boiling with the addition of sodium sulphate and finally each brought to a volume of 100 c. c. 25 c. c. of the saccharose solution, which had been in contact with the stomach tissue, gave no reduction whatever with Fehling's solution. 25 c. c. of the saccharose-intestine solution, however, gave 0·0726 gram Cu, equivalent to 0·0371 gram dextrose.

With the glucose solutions, the following results were obtained:

STOMACH.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0906 gram Cu = 0·0462 gram dextrose × 4 = 0·1848 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0889 gram Cu = 0·0454 gram dextrose × 4 = 0·1816 gram dextrose.

INTESTINE.

*a. Before treatment with sulphuric acid.*

25 c. c. gave 0·0712 gram Cu = 0·0364 gram dextrose × 4 = 0·1456 gram dextrose.

*b. After treatment with sulphuric acid.*

25 c. c. gave 0·0689 gram Cu = 0·0353 gram dextrose × 4 = 0·1412 gram dextrose.

With glucose, the same results are to be observed here as in the preceding experiments; the only variations in reducing power, before and after treatment with sulphuric acid, being such as would come within the ordinary limits of error. In one single case, a transforma-

tion of saccharose was noticed when the sugar solution was warmed for two hours with a portion of stomach tissue from a rabbit killed in full digestion. The reduction with Fehling's solution was quite strong.

With glucose, however, many experiments have been tried in addition to those given above, and invariably with the same negative result. The conditions of the experiments, moreover, are in many cases identical with those of Pavy's except in the method of determining reducing power. There seems, therefore, to be no plausible explanation of the results obtained, other than that in the above experiments there was no dehydrating ferment present. Pavy states that the ferment in question, or rather "the active principle concerned in the transformation of glucose is susceptible of being destroyed by the agency of gastric digestion," so that there is the possibility of such destructive action having taken place in the stomach of the animals experimented with. It is further stated, however, that the converting principle is situated in the underlying portion of the mucous membrane, so that destruction could hardly be expected, except perhaps in the slow self-digestion occurring after death. Certainly, the carefully rinsed tissue could not have retained sufficient gastric juice to affect the results. Furthermore, such decomposition would apply only to the stomach mixture and not to the intestines, unless sufficient proteolytic ferment from the pancreatic juice should adhere to the walls of the intestines to exert destructive action; but in the last experiment given, it is to be noticed that the saccharose ferment, which is presumably equally sensitive, showed vigorous action while the glucose was unaffected.

It seems strange, therefore, if such a dehydrating ferment is normally present in the alimentary tract, that we have not been able to obtain some tangible evidence of its presence, either in the stomach or intestines.

Since the above was written, the writer has noticed that M. Ogáta,* experimenting with dogs, has also been unable to confirm Pavy's results, both in the dehydration of glucose and in the inversion of saccharose.

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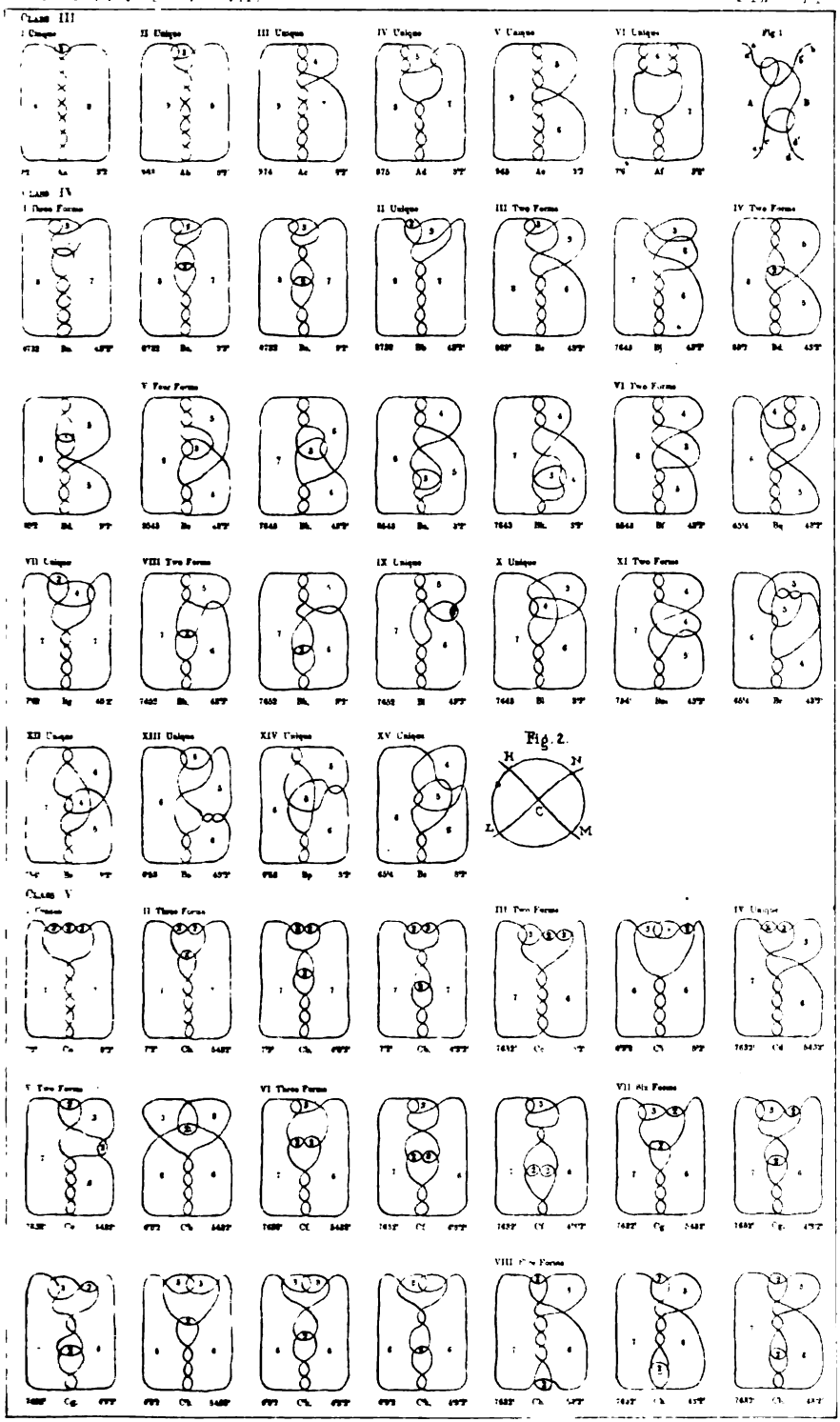
*See Jahresbericht für Thierchemie, xv, 275.


Class III
I Unique
II Unique
III Unique
IV Unique
V Unique
VI Unique
Fig 1
Class IV
I Three Forms
II Unique
III Two Forms
IV Two Forms
V Two Forms
VI Two Forms
VII Unique
VIII Two Forms
IX Unique
X Unique
XI Two Forms
XII Unique
XIII Unique
XIV Unique
XV Unique
Fig. 2.
H
N
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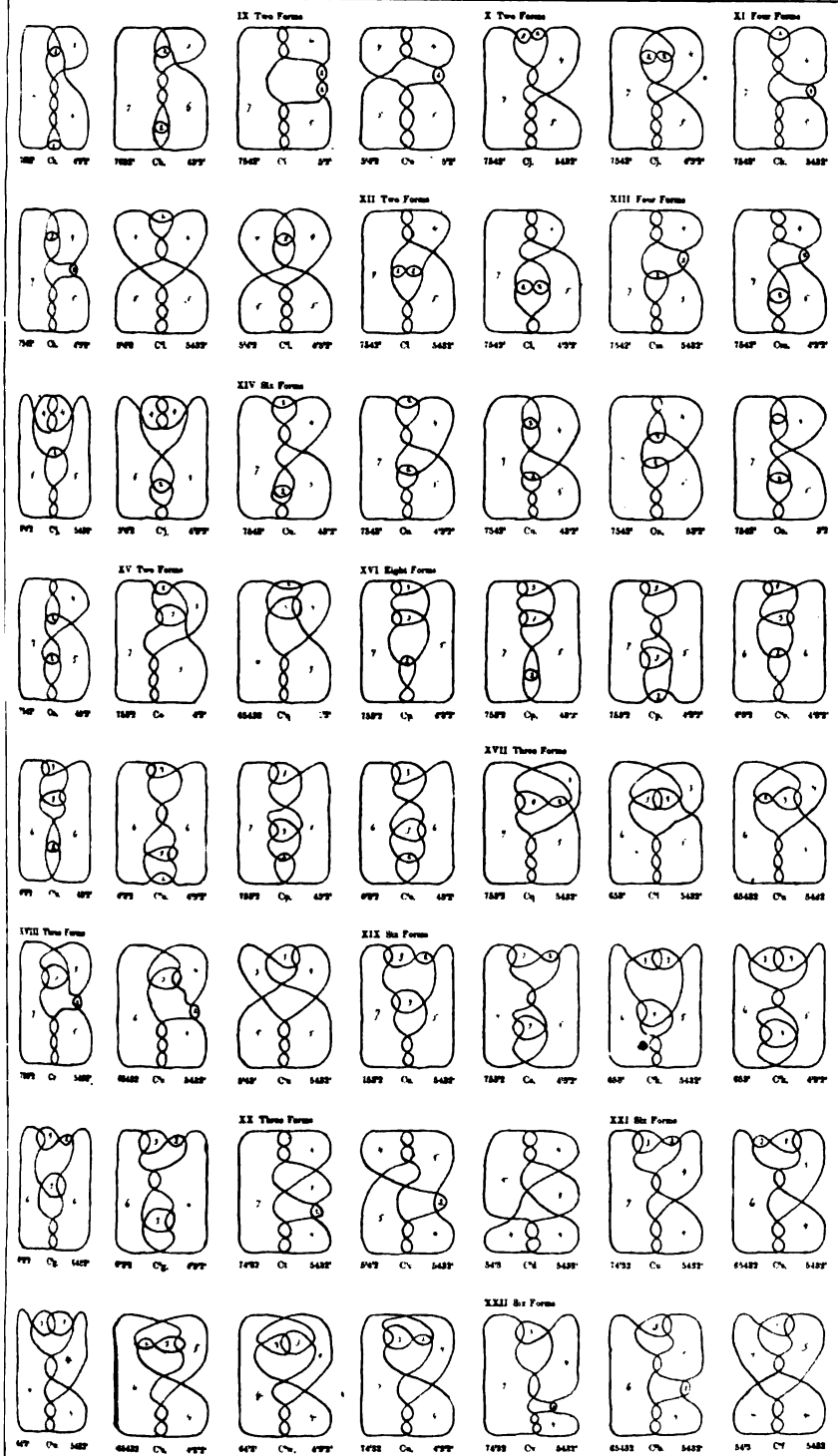
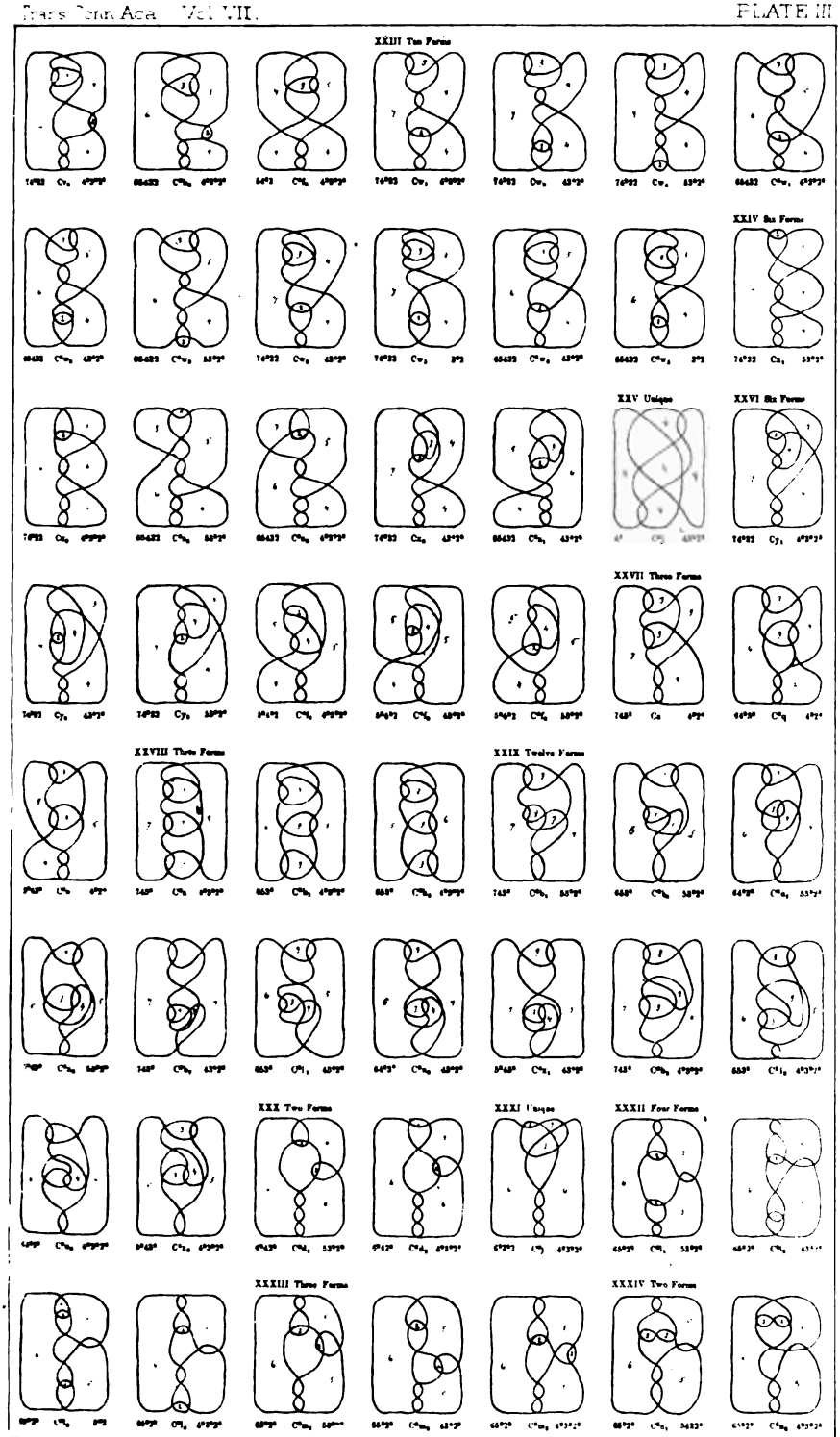
Class V
I Unique
II Three Forms
III Two Forms
IV Unique
V Two Forms
VI Three Forms
VII Six Forms
VIII Six Forms


IX Two Forms
X Two Forms
XI Four Forms
XII Two Forms
XIII Four Forms
XIV Six Forms
XV Two Forms
XVI Eight Forms
XVII Three Forms
XVIII Three Forms
XIX Six Forms
XX Three Forms
XXI Six Forms
XXII Six Forms

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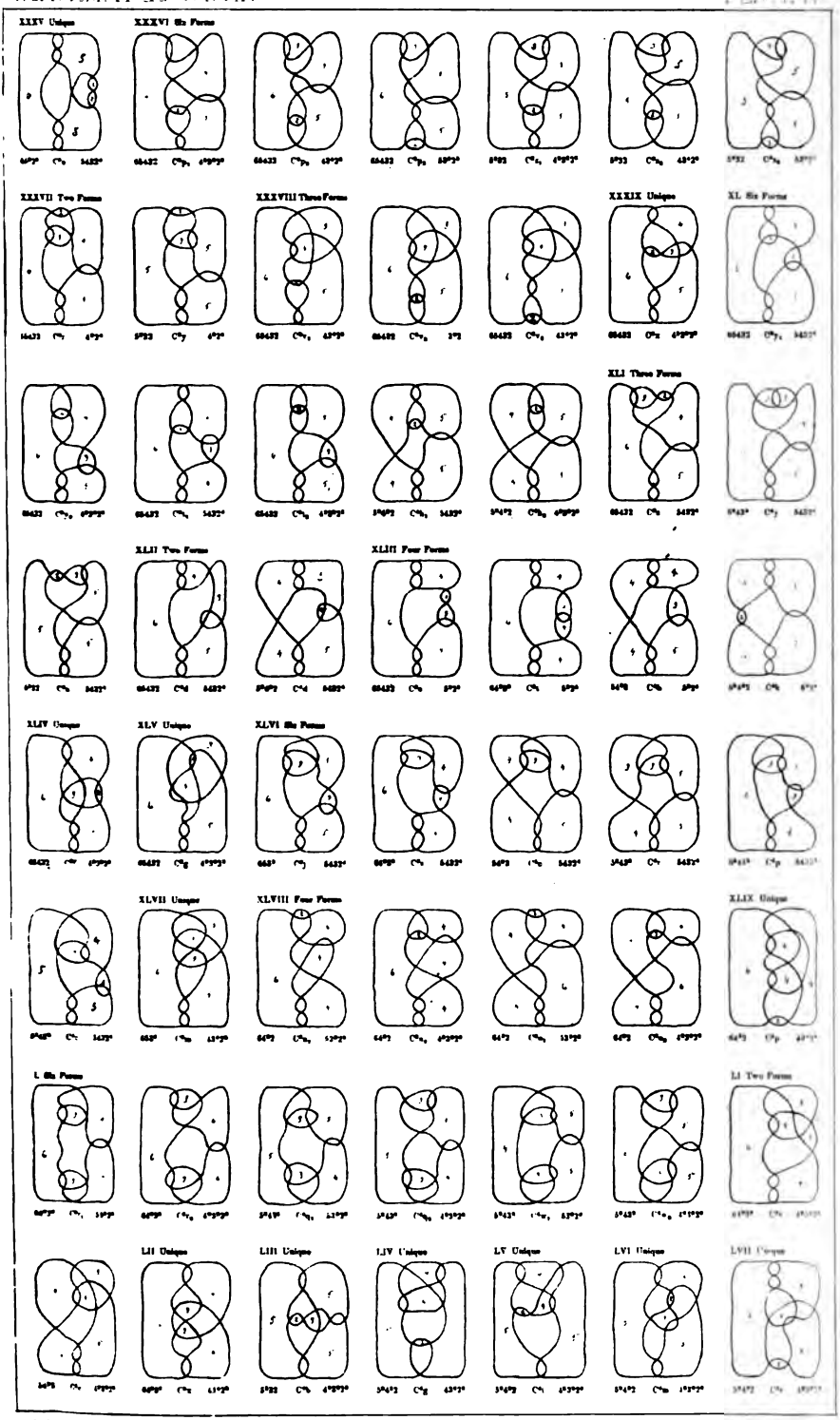
XXXV Unique
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XXXVIII Three Forms
XXXIX Unique
XL Six Forms
XLI Three Forms
XLII Two Forms
XLIII Four Forms
XLIV Unique
XLV Unique
XLVI Six Forms
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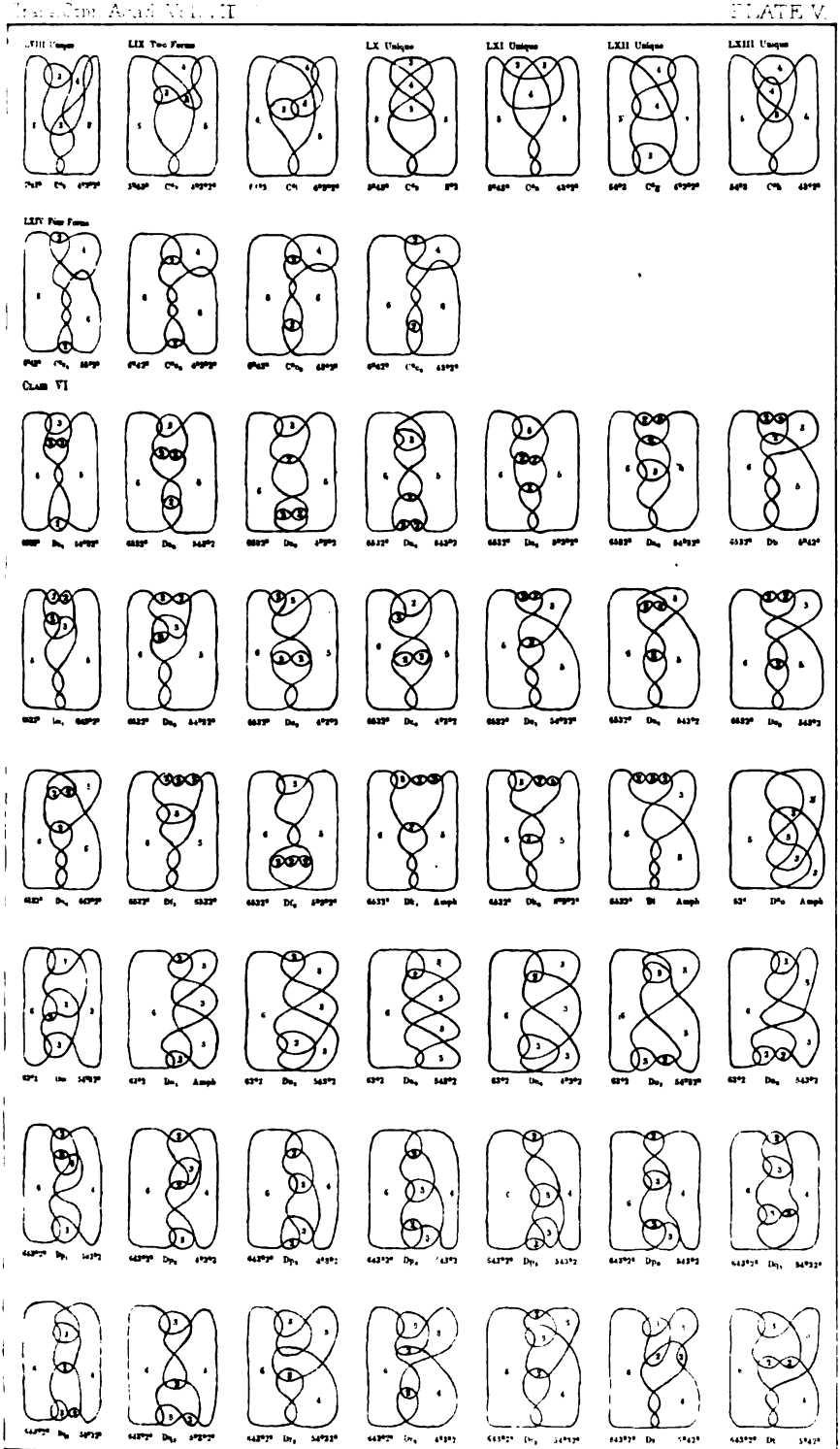
LVII Unique

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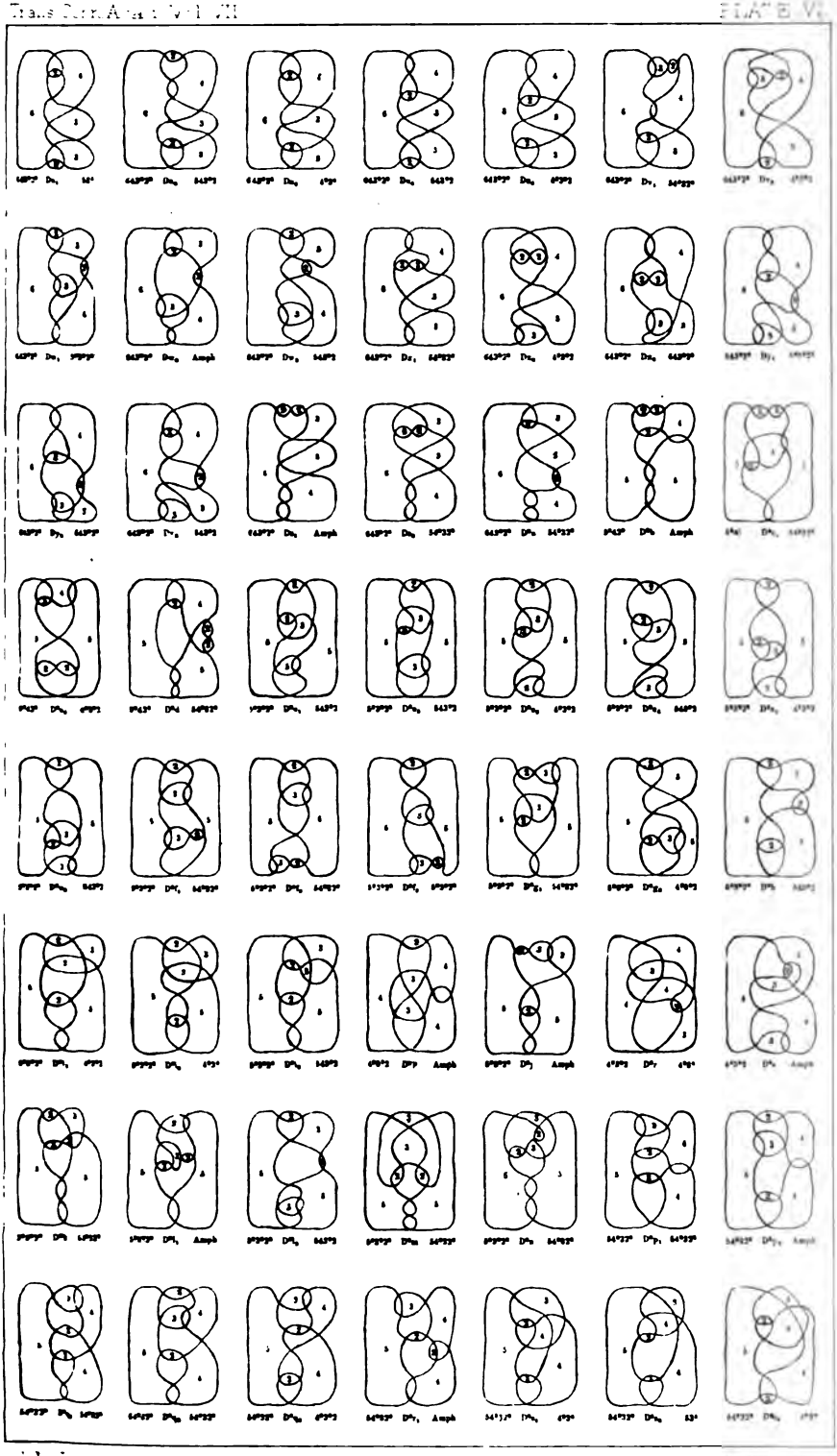

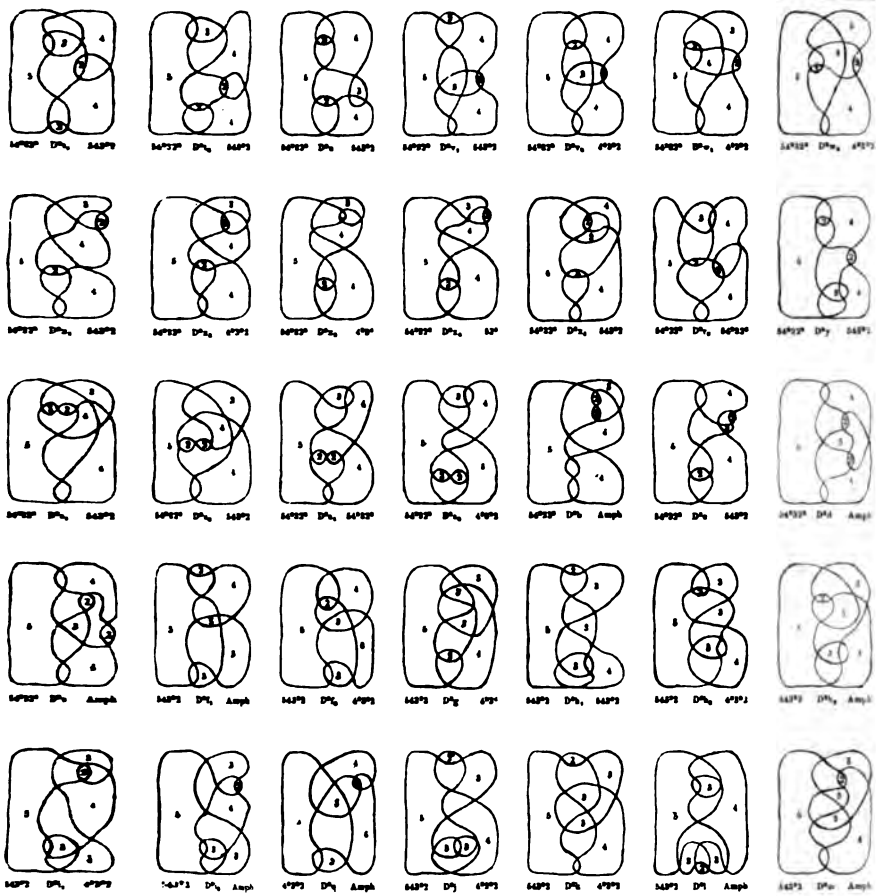

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BASEL.—*Naturforschende Gesellschaft.*
Verhandlungen. Theil VIII. 1, 2, 1886-87. 8°.
BATAVIA.—*Kon. Natuurkundige Vereeniging in Nederlandsch-Indië.*
Natuurkundige tijdschrift. Deel XLVI, XLVII, 1887. 8°.
——*Magnetical and Meteorological Observatory.*
Observations. Vol. VI supplement, VII, IX, 1886-87. 4°.
BERGEN.—*Museum.*
Aarsberetning. 1886. 8°.
BERLIN.—*Königliche Sternwarte.*
Berliner astronomisches Jahrbuch. 1889, 1890. 8°.
BOLOGNA.—*R. Accademia delle Scienze dell' Istituto di Bologna.*
Rendiconto. Anno 1886-87. 8°.
BOMBAY.—*Bombay Branch of the Royal Asiatic Society.*
Journal. No. XLV, XLVI, 1887. 8°.
Index to Transactions vol. I-III, and Journal vol. I-XVII. 1886. 8°.
——*Government Observatory.*
Magnetical and meterological observations. 1885. 4°.
BONN.—*Naturhistorischer Verein der preussischen Rheinlande, Westfalens und des Reg.-Bezirks Osnabrück.*
Verhandlungen. Jahrg. XLIII. 2, XLIV, 1886-87. 8°.
BORDEAUX.—*Académie Nationale des Sciences, Belles-Lettres et Arts.*
Actes. Année XLVII, 1885. 8°.
——*Société Linnéenne.*
Actes. Tome XXXIX, 1885. 8°.
Procès-verbaux. 1886. 8°.
——*Société des Sciences Physiques et Naturelles.*
Mémoires. 3e sér. Tome II. 2, appendice iii, III. 1, 1886. 8°.
BRAUNSCHWEIG.—*Verein für Naturwissenschaft.*
Jahresbericht. III, IV, 1881-86. 8°.
BREMEN.—*Naturwissenschaftlicher Verein.*
Abhandlungen. Bd. IX. 4, X. 1, 2, 1887-88. 8°.
BRESLAU.—*Schlesische Gesellschaft für vaterländische Cultur.*
Jahres-Bericht. LXIII u. Ergänz., LXIV u. Ergänz., 1885-86. 8°.
BRÜNN.—*Natürforscher Verein.*
Verhandlungen. Bd. XXIV, XXV, 1885-86. 8°.
Bericht der meteorologischen Commission. V, 1885. 8°.

BRUXELLES.—*Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique*
Mémoires. Tome XLVI, 1886. 4°.
Mémoires couronnés et mémoires des savants étrangers. Tome XLVII, XLVIII, 1886. 4°.
Mémoires couronnés et autres mémoires. Tome XXXVII—XXXIX, 1886. 8°.
Bulletins. 3e sér. Tome IX-XII, 1885-86. 8°.
Annuaire. Année LII, LIII, 1886-87. 8°.
Notices biographiques et bibliographiques. 1886. 8°.
Catalogue des livres de la bibliothèque. Partie I, II. 1, 2. 1881-87. 8°.
——*Musée Royal de l'Histoire Naturelle.*
Bulletin. Tome IV. 4, 1886. 8°.
——*Société Entomologique de Belgique.*
Annales. Tome XXX, 1886. Table générale, tome I-XXX. 1887. 8°.
——*Société Royale Belge de Géographie.*
Bulletin. Année X. 2-6, XI, 1886-87. 8°.
——*Société Royale de Botanique.*
Bulletin. Tome XXV. 3, XXVI. 1, 1886-87. 8°.
——*Société Royale Malacologique de Belgique.*
Annales. Tome XXI, 1886. 8°.
Procès-verbaux. Tome XV pp. 97-143, XVI pp. 1-80, 1886-87. 8°.
Statuts. 2e éd. 1886. 8°.
BUCAREST.—*Institut Météorologique de Roumanie.*
Annales. Tome I, II, 1885-86. 4°.
Serviciulŭ meteorologicŭ in Europa. Note de călĕtoriă de inginerŭ St. C. Hepites. 1884. 4°.
BUDAPEST.—*Kön. ung. Central-Anstalt für Meteorologie und Erdmagnetismus.*
Jahrbücher. Jahrg. XV, 1885. 4°.
BUENOS AIRES.—*Museo Publico.*
Anales. Entrega XIII, XIV, 1883-85. 4°.
Atlas de la description physique de la République Argentine. Par H. Burmeister. Section II. Mammifères. Livr. 1-3, 1881-86. f°.
——*Sociedad Cientifica Argentina.*
Anales. Tome XXII. 5, 6, XXIII, XXIV, XXV. 1, 1886-88. 8°.
Estudio critico y comparativo de las reglas de Descartes y de Newton respecto al numero de raices de las ecuaciones numéricas. Por Arturo Orzabel. 1886. 8°.
CAEN.—*Société Linnéenne de Normandie.*
Bulletin. 4e sér. Vol. I, 1886-87. 8°.
CALCUTTA.—*Asiatic Society of Bengal.*
Journal. Vol. LV, pt. i, no. 3, 4; pt. ii, no. 3-5; LVI, pt. i, no. 1-3; pt. ii; LVII, pt. ii, no. 1; 1886-88. 8°.
Proceedings. 1886, no. 8-10, 1887, 1888, no. 1-3. 8°.
Descriptions of new Indian Lepidopterous insects from the collection of the late Mr. W. S. Atkinson. Pt. III, 1888. 4°.
——*Geological Survey of India.*
Palæontologia Indica. Ser. I, vol. i, pt. 1, (reprint); X, vol. iv, pt. 3, XII, vol. iv, pt. 2; XIII, vol. i, pt. 6; 1886-87. 4°.
Memoirs Vol. XXIV. 1, 1887. 8°.
Records. Vol. XX, XXI. 1, 2, 1887-88. 8°.
Manual of the geology of India. Pt. IV. Mineralogy. 1887. 8°.
Catalogue of the remains of Siwalik Vertebrata in the Indian museum, Calcutta. Pt. 1, Mammalia. Pt. 2, Aves, Reptilia and Pisces. Pleistocene and pre-historic Vertebrata. 1885-86. 8°.
——*Meteorological Department of the Government of India.*
Indian meteorological memoirs. Vol. IV. 2-4, 1884-86. f°.

CALCUTTA.—*Meteorological Department of the Government of India.*

Report on the meteorology of India. 1885, 1886. f°.

Report on the administration of the meteorological department. 1885-86, 1886-87. f°.

Meteorological observations recorded at six stations in India. 1886 July-Dec., 1887. f°.

Cyclone memoirs. Pt. I, 1887. 8°.

Charts of the Bay of Bengal and adjacent sea north of the equator showing the mean pressure, winds and currents in each month of the year. 1886. f°.

—— —— showing the specific gravity, temperature and currents of the sea surface. 1887. f°.

Memoirs of the winds and monsoons of the Arabian Sea and North Indian Ocean. By W. L. Dallas. 1887. 4°.

CAMBRIDGE.—*Philosophical Society.*

Transactions. Vol. XIV. 1, 2, 1885-87. 4°.

Proceedings. Vol. V. 6, VI. 1-3, 1886-87. 4°.

CASSEL.—*Verein für Naturkunde.*

Bericht. XXXII-XXXIII, 1884-86. 8°.

CHERBOURG.—*Société Nationale des Sciences Naturelles.*

Mémoires. Tome XXV, 1887. 8°.

CHRISTIANIA.—*Kong. Norske Universitet.*

Norges væxtrige. Af Dr. F. C. Schübeler. Bd. I. 2, II. 1. 1886. 4°.

Joannis Agricolæ Islebiensis apothegmata nonnulla nunc primum edidit Dr. L. Daae. 1886. 4°.

——*Norwegisches meteorologisches Institut.*

Jahrbuch. 1885. 4°.

——*Norwegian North-Atlantic Expedition*, 1876-78.

Publication XVI-XVIII. 1886-87. 4°.

——*Videnskabs Selskabet.*

Forhandlingar. 1886. 8°.

CHUR.—*Naturforschende Gesellschaft Graubündens.*

Jahres-Bericht. Neue Folge. Jahrg. XXIX, XXX, 1884-85, 1885-86. 8°.

CORDOBA.—*Academia Nacional de Ciencias.*

Actas. Tomo V. 3, 1886. 4°.

Boletin. Tomo IX, X. 1, 1886-87. 8°.

DANZIG.—*Naturforschende Gesellschaft.*

Schriften. Neue Folge. Bd. IV. 4, VI. 4, 1878-84. 8°.

DIJON.—*Académie des Sciences, Arts et Belles-Lettres.*

Mémoires. 3e sér. Tome IX, 1885-86. 8°.

DORPAT.—*Gelehrte Estnische Gesellschaft.*

Sitzungsberichte. 1886. 8°.

——*Naturforscher-Gesellschaft bei der Universität Dorpat.*

Archiv für die Naturkunde Liv-Ehst-und Kurlands. Ser. I. Bd. IX. 4, 1887. 8°.

Sitzungsberichte. Bd. VIII. 1, 2, 1886-87. 8°.

Schriften. II-IV, 1887-88. 8°.

DRESDEN.—*Naturwissenschaftliche Gesellschaft Isis.*

Sitzungsberichte und Abhandlungen. 1885 Juli-Dec., 1886, 1887. 8°.

DUBLIN.—*Royal Irish Academy.*

Transactions. Vol. XXVI. 22, XXVII. 6-8, XXVIII. 21-25, 1879-86. 4°.

Proceedings. Ser. II. Science. Vol. IV. 5, 1886. 8°.

Cunningham memoirs. No. I-IV, 1880-87. 4°.

EDINBURGH.—*Geological Society.*

Transactions. Vol. V. 2, 3, 1887. 8°.

Catalogue of the library. 1887. 8°.

EDINBURGH.—*Royal Physical Society.*
Proceedings. Vol. IX. 1, 1885–86. 8°.
——*Royal Society.*
Proceedings. Vol. XII pp. 245–1008, XIII, XIV, 1883–87. 8°.
List of members. Nov. 1887. 4°.
EMDEN.—*Naturforschende Gesellschaft.*
Jahresbericht. LXXI, 1885–86. 8°.
ERFURT.—*Kön. Akademie gemeinnütziger Wissenschaften.*
Jahrbücher. Neue Folge. Heft XIV, 1886. 8°.
FIRENZE.—*Biblioteca Nazionale Centrale.*
Bollettino delle pubblicazioni Italiane ricevute per diritto di stampa. No. 25–61, 1887–88. 8°.
FRANKFURT A. M.—*Deutsche malakozoologische Gesellschaft.*
Nachrichtsblatt. Jahrg. XVIII. 11, 12, XIX, XX. 1–6, 1886–88. 8°.
——*Senckenbergische naturforschende Gesellschaft.*
Abhandlungen. Bd. XIV. 2, 3, XV. 1, 2, 1886–88. 4°.
Bericht. 1886, 1887. 8°.
FREIBURG IN B.—*Naturforschende Gesellschaft.*
Verhandlungen. Bd. VIII. 3, 1885. 8°.
Berichte. Bd. I, 1886. 8°.
GENÈVE.—*Institut National Genevois.*
Bulletin. Tome XXVII, XXVIII, 1885–88. 8°.
Mémoires. Tome XVI, 1883–86. 4°.
——*Société de Physique et d'Histoire Naturelle.*
Mémoires. Tome XXIX. 2, 1886–87. 4°.
GIESSEN.—*Oberhessische Gesellschaft für Natur-und Heilkunde.*
Bericht. XXV, 1887. 8°.
GLASGOW.—*Natural History Society.*
Proceedings and transactions. New series. Vol. I. 3, 1885–86. 8°.
——*Philosophical Society.*
Proceedings. Vol. XVII, XVIII, 1885–86, 1886–87. 8°.
GÖRLITZ.—*Naturforschende Gesellschaft.*
Abhandlungen. Bd. XIX, 1887. 8°.
GÖTTINGEN.—*Königl. Gesellschaft der Wissenschaften.*
Nachrichten. 1886, 1887. 8°.
GÜSTROW.—*Verein der Freunde der Naturgeschichte in Mecklenburg.*
Archiv. Jahrg. XL, XLI, 1886–87. 8°.
HABANA.—*Real Colegio de Belen.*
Observaciones magneticas y meteorologicas. 1885 iv, 1886 i–iii. 4°.
HALIFAX.—*Department of Mines.*
Report. 1887. 8°.
HALLE.—*Kais. Leopoldinisch-Carolinische deutsche Akademie der Naturforscher.*
Leopoldina. Heft XXII, XXIII, 1886–87. 4°.
——*Naturforschende Gesellschaft.*
Abhandlungen. Bd. XVI. 4, 1886. 4°.
Bericht. 1885, 1886. 8°.
——*Naturwissenschaftlicher Verein für Sachsen und Thüringen.*
Zeitschrift für die gesammten Naturwissenschaften. Bd. LIX. 3–6, LX, 1886–87. 8°.
HAMBURG.—*Deutsche Seewarte.*
Archiv. Jahrg. VIII, IX, 1885–86. 4°.
Monatliche Uebersicht der Witterung. 1886, 1887, 1888 Januar, Februar. 8°.

HAMBURG.—*Naturwissenschaftlicher Verein.*
Abhandlungen. Bd. IX, X, 1886–87. 4°.
——— *Wissenschaftliche Anstalten.*
Jahrbuch. Jahrg. IV, V, 1886–87.
HANNOVER.—*Naturhistorische Gesellschaft.*
Jahresbericht. XXXIV–XXXVII, 1883–1887. 8°.
HARLEM.—*Musée Teyler.*
Archives. Série II. Vol. III. 1, 1887. 8°.
Catalogue de la bibliothèque. Livr. 5, 6. 1886. 8°.
Liste alphébetique de la correspondence de Christian Huyghens. 4°.
———*Société Hollandaise des Sciences.*
Archives néerlandaises des sciences exactes et naturelles. Tome XXI. 2–5, XXII, 1886–87. 8°.
HELSINGFORS.—*Societas Scientiarum Fennica.*
Öfversigt af förhandlingar. XXVII, 1884–85.
Bidrag till kännedom af Finlands natur och folk. Häft. XLIII, XLIV, 1886–87. 8°.
Exploration internationale des régions polaires, 1882–83 et 1884–85. Expédition polaire finlandaise. Tome I, II. 1886–87. 4°.
———*Institut Météorologique Central.*
Observations. Vol. I, II. 1, 1882–83. 4°.
HONGKONG.—*Observatory.*
Observations and researches. 1886. f°.
JENA.—*Medicinisch-naturwissenschaftliche Gesellschaft.*
Jenaische Zeitschrift für Naturwissenschaft. Bd. XX, XXI, XXII. 1, 2, 1887–88. 8°.
KIEL.—*Königl. Christian Albrechts-Universität.*
Dissertationen, etc. (48). 1886–87.
———*Naturwissenschaftlicher Verein für Schleswig-Holstein.*
Schriften. Bd. VII. 1, 1888. 8°.
KIEV.—*Kievskie Obshchestvo Iestestvoispytateleï.*
Zapiski. Tom. VII, VIII and suppt., 1883–87. 8° and 4°.
KJÖBENHAVN.—*Kon. Danske Videnskabernes Selskab.*
Oversigt over forhandlinger. 1886 ii, iii, 1887 i, ii. 8°.
KÖNIGSBERG.—*Königl. physikalisch-ökonomische Gesellschaft.*
Schriften. Jahrg. XXVII, 1887. 4°.
KRAKÓW.—*K. k. Sternwarte.*
Materyaly do klimatografii Galicyi. Rok 1886. 8°.
LAUSANNE.—*Société Vaudoise des Sciences Naturelles.*
Bulletin. 3e sér. No. 94–96, 1886–87. 8°.
LEEDS.—*Yorkshire Geological and Polytechnic Society.*
Proceedings. New series. Vol. IX. 2, 3, 1886–88. 8°.
LEIDEN.—*Nederlandsche Dierkundige Vereeniging.*
Tijdscrift. Ser. II. Deel I. 3, 4, II. 1, 2, 1886–88. 8°.
LEIPZIG.—*Astronomische Gesellschaft.*
Vierteljahrsschrift. Jahrg. XXII, 1887. 8°.
Publication XVIII. 1886. 4°.
———*Kön. sächsische Gesellschaft der Wissenschaften.*
Berichte. Math.-physische Classe. Bd. XXXVIII, XXXIX, 1886–87. 8°.
———*Verein für Erdkunde.*
Mittheilungen. 1884–1886. 8° and f°.
———Zoologischer Anzeiger. No. 241–282, 1887–88. 8°.
LIÉGE.—*Société Géologique de Belgique.*
Annales. Tome XIII. 1, 1887. 8°.
Procès-verbal de l'assemblée générale du 21 Nov. 1886. 8°.

LIÉGE.—*Société Royale des Sciences.*
Mémoires. 2e sér. Tome XIII, XIV, 1886–88. 8°.
LINZ.—*Museum Francisco-Carolinum.*
Bericht. XLIV, XLV, 1886. 8°.
LISBOA.—*Sociedade de Geographia.*
Boletim. Serie VI. 7–12, VII. 1–8, 1886–87. 8°.
Elogio historico do Antonio Augusto d'Aguiar. Por Gomez de Brito. 1887. 8°.
LIVERPOOL.—*Literary and Philosophical Society.*
Proceedings. No. XXXIX, XL, 1884–86. 8°.
LONDON.—*Geological Society.*
Quarterly journal. Vol. XLIII, XLIV. 1, 2, 1887–88. 8°.
List. 1887. 8°.
——*Linnean Society.*
Journal. Zoology. No. 114–117, 126–29, 1886–87. 8°.
Botany. No. 145–149, 151, 158, 1886–87. 8°.
Proceedings. 1883–87. 8°.
List. 1886–87. 8°.
——*Mathematical Society.*
Proceedings. No. 273–320, 1886–88. 8°.
List of members. 1887. 8°.
——*Royal Meteorological Society.*
Quarterly journal. New series. No. 61–66, 1886–88. 8°.
List of fellows, March 1, 1888. 8°.
——*Royal Historical Society.*
Transactions. New series. Vol. III. 3, 4, 1886. 8°.
England and Napoleon in 1803, being the despatches of Lord Wentworth and others. Edited by Oscar Browning. 1887. 8°.
The teaching of history in schools. An address by Oscar Browning. 1887. 8°.
——*Royal Microscopical Society.*
Journal. 1887, 1888 i–iii, 8°.
——*Royal Society.*
Philosophical transactions. Vol. CLXXVII, 1886–87. 4°.
Proceedings. No. 248–269, 1886–88. 8°.
List of council and members. 1886. 4°.
——*Zoological Society.*
List of vertebrated animals now or lately living in the gardens of the Zoological Society of London. 8th ed. 1883. 8°.
LUND.—*Universitet.*
Acta. Tom. XXII, XXIII, 1885–87. 4°.
LUXEMBOURG.—*Institut Royal Grand-Ducal.*
Publications. Section des sciences mathématiques et naturelles. Tome XX, 1886. 8°.
Observations météorologiques. Vol. III, IV, 1887. 8°.
LYON.—*Musée Guimet.*
Annales. Tome X–XII, 1886–87. 4°.
Revue de l'histoire des religions. Tome XIV, 2, 3, XV, XVI. 1, 1886–87.
Sécurité dans les théatres. Par M. Émile Guimet. 1887. 8°.
LYME REGIS.—*Roussdon Observatory.*
Publications. Vol. IV. Meteorological observations for 1887. 4°.
MADRAS.—*Government Observatory.*
Observations of the fixed stars made with the meridian circle, 1862–67. 4°.

MADRID.—*Comision del Mapa Geologico de España.*
Boletin. Tomo XII. 2, XIII. 2, 1885–86. 8°.
Memorias. Descripcion fisica y geologica de la provincia de Alava. Por D. Ramon Adan de Yarza. 1885. 8°.
———Descripcion fisica y geologica de la provincia de Zamora. Por D. Gabriel Puig y Larraz. 1883. 8°.
———*Observatorio.*
Observaciones meteorologicas. 1882–83, 1884–85. 8°.
Resumen da las observacionés efectuadas en la peninsula. 1883. 8°.
———*Sociedad Española de Historia Natural.*
Anales. Tomo XV. 3, XVI, XVII. 1, 1886–88. 8°.
———*Real Academia de Ciencias Exactas, Fisicas y Naturales.*
Memorias. Tomo XI, XII, XIII. 1, 1887. 4°.
Revista de los progresos de las ciencias exactas. Tomo XXI. 7–9, XXII. 1–4, 1886–87. 8°.
Anuario. 1888. 8°.
MAGDEBURG.—*Naturwissenschaftlicher Verein.*
Jahresbericht und Abhandlungen. 1887. 8°.
Das Innere der Erde. Vortrag von Dr. phil. Ernst Heintzmann am 8. Mai, 1888. 8°.
MANCHESTER.—*Literary and Philosophical Society.*
Memoirs. Series III. Vol. X, 1887. 8°.
Proceedings. Vol. XXV, XXVI, 1885–87. 8°.
MARBURG.—*Gesellschaft zur Beförderung der gesammten Naturwissenschaften.*
Sitzungsberichte. Jahrg. 1886, 1887. 8°.
MELBOURNE.—*National Museum.*
Prodromus of the zoology of Victoria. Decade I–XV. 1878–87. 8°.
METZ.—*Académie.*
Mémoires. 3e sér. Année XIII, 1883–84. 8°.
MEXICO.—*Observatorio Meteorologico-Magnetico Central.*
Boletin mensuel. Tomo I. 1–5, 1888. 8°.
———*Museo Nacional.*
Anales. Tomo III. 11, IV. 1, 2, 1886–88. 4°.
———*Sociedad Cientifica "Antonio Alzate."*
Memorias. Tomo I. 1–5, 8–10, 12, 1887–88. 8°.
———*Sociedad de Geographia y Estadistica.*
Boletin. Epoca III. Tomo VI. 4–9, 1887. 8°.
———*Sociedad Mexicana de Historia Natural.*
La naturaleza. Tomo VII. 19–24. Ser. II. Tomo I. 1–3. 1886–88. 4°.
MILANO.—*Real Istituto Lombardo di Scienze e Lettere.*
Rendiconto. Serie II. Vol. XVIII, XIX, 1885–86. 8°.
———*Real Osservatorio di Brera.*
Pubblicazioni. No. VII. 3, XXVII–XXXII, 1885–87. 4°.
MODENA.—*Regia Accademia delle Scienze, Lettere ed Arti.*
Memorie. Tomo XX. 3. Serie II. Tomo IV. 1886. 4°.
———*Società dei Naturalisti.*
Memorie. Ser. III. Vol. V. VI, 1886–87. 8°.
Rendiconti. Ser. III. Vol. III pp. 1–128, 1886. 8°.
MONTPELLIER.—*Académie des Sciences et Lettres.*
Mémoires. Section des lettres. Tome VIII. 1, 1886–87. 4°.
———Section des sciences. Tome XI. 1, 1885–86. 4°.
MONTREAL.—*Natural History Society.*
The Canadian record of science. Vol. II. 5, 6, 1887. 8°.
MOSCOU.—*Société Impériale des Naturalistes.*
Bulletin. Année 1886, 1887. 8°.

MOSCOU.—*Société Impériale des Naturalistes.*

Meteorologische Beobachtungen am Observatorium der landwirth. Akademie bei Moskau. Jahr. 1886 ii, 1887 i. 4°.

MÜNCHEN.—*Kön. bayerische Akademie der Wissenschaften.*

Sitzungsberichte. Philosph.-philolog. und histor. Classe. 1886, 1887 Bd. I, II. 1-3. 8°.

——Mathemat.-physikal. Classe. 1886, 1887 Heft 1-3. 8°.

——Inhaltsverzeichniss. Jahrg. 1876-1885. 8°.

Gedächtnisrede auf Joseph v. Fraunhofer. Von Carl Max v. Bauernfeind. 1887. 4°.

Gedächtnissrede auf Carl Theodor v. Siebold. Von Richard Hertwig. 1886. 4°.

Gedächtnissrede auf Leopold v. Ranke. Von W. v. Giesebrecht. 1887. 4°.

Ueber historische Dramen der Roemer. Festrede von Dr. Karl Meiser. 1887. 4°.

MÜNSTER.—*Westfälischer Provincial-Verein für Wissenschaft und Kunst.*

Jahresbericht. XIV, 1885. 8°.

Die Kunst- und Geschichts-Denkmäler der Provinz Westfalen. Stück II; Kreis Warendorf. 1886. 4°.

NANCY.—*Académie de Stanislas.*

Mémoires. 5e sér. Tome IV, 1887. 8°.

NAPOLI.—*R. Accademia delle Scienze Fisiche e Matematiche.*

Rediconto. Anno XXV. 4-12, 1886. 4°.

——*Zoologische Station.*

Mittheilungen. Bd. VII, 1887. 8°.

NEUCHATEL.—*Société des Sciences Naturelles.*

Bulletin. Tome XV, 1886. 8°.

NEWCASTLE-UPON-TYNE.—*North of England Institute of Mining and Mechanical Engineers.*

Transactions. Vol. XXXVI, XXXVII. 1-4, 1886-87. 8°.

NÜRNBERG.—*Naturhistorische Gesellschaft.*

Jahresbericht. 1886, 1887. 8°.

Festschrift zur Begrüssung des xviii. Kongresses der Deutschen Anthropologischen Gesellschaft in Nürnberg, 1887. 8°.

ODESSA.—*Société des Naturalistes de la Nouvelle Russie.*

Zapiski. Tom. XI. 2, XII. 1, 1887. 4°.

Matematicheskoe otdielenie. Tom. VII, 1886. 8°.

OTTAWA.—*Geological and Natural History Survey of Canada.*

Annual report. New series. Vol. I, II, with maps, 1885-86. 8°.

——*Meteorological Service of the Dominion of Canada.*

Report. 1883, 1884. 8°.

OXFORD.—*Radcliffe Library.*

Catalogue of books added during 1887. 8°.

Catalogue of transactions of societies, periodicals and memoirs. 4th. ed. 1887. 8°.

——*Radcliffe Observatory.*

Results of astronomical and meteorological observations. Vol. XXXVIII-XLII, 1880-88. 8°.

PALERMO.—*R. Accademia di Scienze, Lettere e Belle Arti.*

Atti. Nuova serie. Vol. IX, 1887. 4°.

Bullettino. 1886 no. 6; 1887 no. 1-6; 1888 Gennaro. 4°.

——*Real Osservatorio.*

Pubblicazioni. Vol. III, 1883-85. 4°.

PARIS.—*École Normale Supérieure.*

Annales scientifiques. 3e sér. Tome I-IV, V. 1-7, 1884-88. 4°.

PARIS.—*École Polytechnique.*
Journal. Cahier LVI, LVII, 1886–87. 4°.
——*Observatoire.*
Rapport annuel. 1887. 4°.
——*Société Nationale d'Acclimatation.*
Bulletin. 3e sér. Tome X. 1, 10, 11. 4e sér. Tome III. 12, IV. 1, 2, 5–7, 11, V. 2, 4, 7–15. 1883–88. 8°.
——*Société Géologique de France.*
Bulletin. 3e sér. Tome XIV. 8, XV. 1–8, XVI. 1–3, 1886–88. 8°.
——*Société Mathématique de France.*
Bulletin. Tome XIV. 5, XV, XVI. 1–3, 1886–88. 8°.
PENZANCE.—*Royal Geological Society of Cornwall.*
Transactions. Vol. XI, 1, 2, 1888–88. 8°.
PISA.—*Società Toscana di Scienze Naturali.*
Memorie. Vol. VIII, 1886–87. 8°.
Processi verbali. Vol. V pp. 118–200, 227–304, VI pp. 59–69, 1886–88. 8°.
PRAG.—*Kön. böhmische Gesellschaft der Wissenschaften.*
Abhandlungen. Folge VII. Bd. I, 1885–86. 4°.
Sitzungsberichte. 1885–1887. 8°.
Jahresbericht. 20 Juli 1885, 16 Januar 1886, 15 Januar 1887. 8°.
Geschichte der kön. böhm. Gesell. d. Wiss. sammt einer kritischen Übersicht ihrer Publicationen aus dem Bereiche der Philosophie, Geschichte und Philologie. Von Joseph Kalousek. Heft. II. 1885. 8°.
Bericht über die mathemat. und naturwiss. Publikationen der kön. böhm. Gesell. d. Wiss. während ihres hundertjährigen Bestandes. Von Dr. F. J. Studnička. Heft. II. 1885. 8°.
——*K. k. Sternwarte.*
Magnetische und meteorologische Beobachtungen. Jahrg. XLVII, XLVIII, 1886–87. 4°.
PUEBLA DE ZARAGOZA.—*Estado de Puebla.*
Boletin de estadistica. Tomo I. 1–44, 1887–88. f°.
PULKOVA.—*Nicolai-Hauptsternwarte.*
Jahresbericht. 1886. 8°.
QUEBEC.—*Literary and Historical Society.*
Transactions. Vol. V. 1, 1862. 8°.
REGENSBURG.—*Naturwissenschaftlicher Verein.*
Correspondenz-Blatt. Jahrg. XL, 1886. 8°.
——*Historischer Verein von Oberpfalz und Regensburg.*
Verhandlungen. Bd. XL, 1886. 8°.
RICHMOND, SURREY.—*Kew Observatory.*
Report of the committee. 1886–87. 8°.
RIGA.—*Naturforscher Verein.*
Correspondenzblatt. Jahrg. XXX, 1887. 8°.
RIO DE JANEIRO.—*Museu Nacional.*
Archivos. Vol. VII, 1887. 4°.
ROMA.—*Biblioteca Nazionale Centrale Vittorio Emanuele.*
Bollettino delle opere moderne straniere acquistate dalle biblioteche pubbliche governative del regno d'Italia. Vol. I. 5, 6, II, 1886–87. 8°.
——*Reale Accademia dei Lincei.*
Atti. Ser. II. Vol. IV, 1875–76. 8°.
Memorie della classe di scienze morali, storiche e filologiche. Ser. III. Vol. XII. Ser. IV. Vol. I. 1883–85. 4°.
Memorie della classe di scienze fisiche, matematiche e naturali. Ser. IV. Vol. I, II, 1884–85. 4°.
Rendiconti. Vol. II. ii. 1, 2, III. i. ii. IV. i. 1–10, 1886–88. 8°.

ROMA.—*Reale Comitato Geologico d'Italia.*
Bollettino. Vol. XVII, 1886. 8°.
——*Società Italiana delle Scienze.*
Memorie di matematica e di fisica. Ser. III. Tomo VI, 1887. 4°.
ROTTERDAM.—*Bataafsch Genootschap der Proefondervindelijke Wijsbegeerte.*
Steven Hoogendijk de stichter van het eerste stoomwerktuig in Nederland. Gedenkrede door den Ingenieur A. Huet, 1887. 4°.
ST. GALLEN.—*Naturwissenschaftliche Gesellschaft.*
Bericht. Jahrg. 1884–85, 1885–86. 8°.
ST. PETERSBURG.—*Hortus Petropolitanus.*
Acta. Tom. X. 1, 1887. 8°.
——*Imp. Russ. Geograf. Obshtchestvo.*
Otchet. God 1886. 8°.
Beobachtungen der russ. Polarstation an der Lenamündung. Theil II. 1, 2, 1886–87. 4°.
Beobachtungen der russ. Polarstation auf Nowaja Semlja. Theil II. 1886. 4°.
——*Kais. Akademie der Wissenschaften.*
Repertorium der Meteorologie. Bd. X; Supplementbd. II–V, with atlas; 1887. 4°.
——*Physikalisches Centralobservatorium.*
Annalen. Jahrg. 1885, 1886. 4°.
SANTIAGO.—*Deutscher wissenschaftlicher Verein.*
Verhandlungen. Heft III–V, 1886–87. 8°.
*Schweizerische naturforschende Gesellschaft.*
Verhandlungen. Jahresversammlung LXIX, LXX, 1886–87. 8°.
STOCKHOLM.—*Entomologisk Forening.*
Entomologisk tidskrift. Arg. VII, VIII, 1886–87. 8°.
STUTTGART.—*Verein für vaterländische Naturkunde in Württemberg.*
Jahreshefte. Jahrg. XLIII, XLIV, 1887–88. 8°.
SYDNEY.—*Government Observatory.*
Results of meteorological observations made in New South Wales during 1885. 8°.
——*Linnean Society of New South Wales.*
Proceedings. Series II. Vol. II. 2, 3, 1887, 8°.
List of the names of contributors to the first series, vol. i–x, 1875–85. 8°.
——*Royal Society of New South Wales.*
Journal and proceedings. Vol. XIX–XXI, 1885–87. 8°.
TACUBAYA.—*Observatorio Astronomico Nacional.*
Anuario. Año VIII, 1888. 8°.
THRONDHJEM.—*Kon. Norske Videnskabers Selskab.*
Skrifter. 1883, 1885. 8°.
TIFLIS.—*Physicalisches Observatorium.*
Magnetische Beobachtungen. 1884–85. 8°.
Meteorologische Beobachtungen. 1885–86. 8°.
TŌKYŌ.—*Imperial University of Japan.*
Journal of the college of science. Vol. I, II. 1–3, 1887–88. 4°.
——*Seismological Society of Japan.*
Transactions. Vol. X–XII, 1887–88. 8°.
TORINO.—*Musei di Zoologia ed Anatomia Comparata.*
Bollettino. No. 19–25, 33–42, 44–48, 1887–88. 8°.
TORONTO.—*Canadian Institute.*
Proceedings. Ser. III. Vol. IV. 2, V, 1887–88.
Annual report. 1886–87. 8°.

TOULOUSE.—*Académie des Sciences, Inscriptions et Belles-Lettres.*
Mémoires. 8e sér. Tome VIII, 1886. 8°.
UPSALA.—*Regia Societas Scientiarum.*
Nova acta. Ser. III. Vol. XIII. 2, 1887. 4°.
UTRECHT.—*Kon. Nederlandsch Meteorologisch Instituut.*
Nederlandsch meteorologisch jaarboek. Jaarg. XXVII. 2, XXXVIII, XXXIX, 1878–87. 4°.
——*Provinciaal Utrechtsch Genootschap van Kunsten en Wetenschappen.*
Verslag van het verhandelde in de algemeene vergadering. 1886. 8°.
Aanteekeningen van het verhandelde in de sectie-vergaderingen. 1886. 8°.
VENEZIA.—*Istituto Veneto di Scienze, Lettere ed Arti.*
Atti. Ser. VI. Vol. III. 9, IV, V. 1–9, 1885–87. 8°.
VICENZA.—*Accademia Olimpica.*
Atti. Vol. XX, 1885. 8°.
WELLINGTON.—*New Zealand Institute.*
Transactions and proceedings. Vol. XIX, 1887. 8°.
WIEN.—*Kais. Akademie der Wissenschaften.*
Sitzungsberichte. Mathemat.-naturwiss. Classe. Abth. I. Bd. XCI. 5, XCII–XCIV, 1885–86. 8°.
——*K. k. Central-Anstalt für Meteorologie und Erdmagnetismus.*
Jahrbücher. Neue Folge. XXII, 1885. 4°.
—— *K. k. geologische Reichsanstalt.*
Abhandlungen. Bd. XI. 2, XII. 1–4, 1886–87. 4°.
Jahrbuch. Bd. XXXVI. 2–4, XXXVII. 1, 2, 1886–87. 8°.
Verhandlungen. Jahrg. 1886, No. 5–18, 1886, 1887, No. 1–9. 8°.
——*K. k. naturhistorisches Hofmuseum.*
Annalen. Bd. I. 3, II, III. 1, 2, 1886–88. 8°.
——*K. k. Sternwarte.*
Annalen. Bd. II–IV, 1884–86. 4°.
——*K. k. zoologisch-botanische Gesellschaft.*
Verhandlungen. Bd. XXXVII, 1887. 8°.
WIESBADEN.—*Nassauischer Verein für Naturkunde.*
Jahrbücher. Jahrg. XL, 1887. 8°.
WÜRZBURG.—*Physikalisch-medicinische Gesellschaft.*
Sitzungsberichte. Jahrg. 1886, 1887. 8°.
ZÜRICH.—*Naturforschende Gesellschaft.*
Vierteljahrschrift. Jahrg. XXX, XXXI. 1, 2, 1885–86. 8°.

---

Review of the data for the study of the pre historic chronology of America. By Daniel G. Brinton, M.D. Salem, 1887. 8°. *From the Author.*
Verities in Verses. 2d ed. London, 1887–88. 8°.
Copyright and patents for inventions. By R. A. Macfie. Edinburgh, 1879–1883. 2 v. 8°. *From the Author.*
Evolution of the faunas of the lower Lias. By Alpheus Hyatt. Salem, 1888. 8°.
Value in classification of the stages of growth and decline, with propositions for a new nomenclature. By Alpheus Hyatt. Salem, 1888. 8°.
*From the Author.*
Campagnes scientifique du yacht monégasque l'*Hirondelle.* 3e année, 1887. Excursions zoologiques dans les îles de Fayal et de San Miguel. Par Jules de Guerne. Paris, 1888. 8°.
Résultats des campagnes scientifiques accomplies sur son yacht, par S. A. le Prince Albert de Monaco. Vol. I. Hydrographie et zoologie. (Programme). Monaco, 1888. 4°. *From His Highness Prince Albert of Monaco.*

Supplementa faunae Coleoptorum in Transsilvania scripsit Alexander Ormay. Nagy-Szeben, 1888. 8°. *From the Author.*

Estudio critico y comparativo de las reglas de Descartes y de Newton respecto al numero de raices de las ecuaciones numericas. Por Arturo Orzabel. Buenos Aires, 1886. 8°. *From the Author.*

Heights of the White Mountains. By Edward C. Pickering. 8°.

Observations on variable stars in 1886. By Edward C. Pickering. 8°. *From the Author.*

Osservazioni astronomiche e fisiche sull' asse di rotazione e sulla topografia del pianeta Marte, fatte nella Reale Specola di Brera in Milano coll' equatoriale di Merz. Memoria terza di G. V. Schiaparelli. Roma, 1886. 4°. *From the Author.*

## XVII.—Influence of Uranium Salts on the Amylolytic Action of Saliva and the Proteolytic Action of Pepsin and Trypsin. By R. H. Chittenden and M. T. Hutchinson, Ph.B.

Little is known regarding the physiological, or even toxical action of the uranium salts. In 1825, Gmelin* reported upon the results of some experiments on the toxic action of uranic nitrate, but aside from the work done at that time, little is known regarding the action of uranium. It is our purpose, therefore, to carry out in this Laboratory, as opportunity offers, a series of experiments on the physiological and toxical action of uranium salts, and we have commenced the work by endeavoring to ascertain the influence of the above salts on the amylolytic and proteolytic action of the ferments occurring in the digestive fluids of the body. In this connection we wish to express our obligations to Professor H. Carrington Bolton, of Trinity College, for his kindness in supplying us with an abundance of chemically pure uranium compounds.

### 1. *Influence on the amylolytic action of saliva.*

The method employed in determining the extent of amylolytic action was much the same as that previously† used by one of us, except that the amounts of reducing substances formed under the different conditions of the experiments, were determined volumetrically by Fehling's solution, instead of by Allihn's gravimetric method. The experiments were made in series, in which one digestion of each series served as a control for comparison.

The volume of each digestive mixture was 100 c. c. and contained 1 gram of perfectly pure potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva and a given percentage of the uranium salt to be experimented with. The mixtures were then warmed at 40° C. for 30 minutes, at the end of which time, further ferment action was stopped by heating the solutions to boiling. The saliva employed in the experiments was human mixed saliva, freshly collected, filtered and made as neutral as possible with 0·2 per cent. hydrochloric acid, then diluted with water in the pro-

* Edinb. Med. Surg. Gaz., xxvi., 136.

† Studies from this Laboratory, vol. i, 1884–5, p. 2 and 53.

portion of 1 : 5. Hence, each digestive mixture contained 2 c.c. of undiluted saliva.

The amount of reducing substances, which for the sake of convenience are calculated as dextrose, were, as already mentioned, determined volumetrically and from the data so obtained, the percentage of starch converted was likewise calculated.

*Uranyl nitrate.*

With this salt the following results were obtained :

| $UO_2(NO_3)_2 + 6H_2O$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4135 gram. | 37·21 per cent. | 100·0 |
| 0·0001 per cent. | 0·4083 | 36·74 | 98·7 |
| 0·0003 | 0·3873 | 34·85 | 93.6 |
| 0·0005 | 0·3698 | 33·28 | 89·4 |
| 0·001 | 0·3612 | 32·50 | 87·3 |
| 0·003 | 0·3131 | 28·17 | 75·5 |

The inhibitory action of the uranyl salt is plainly manifest in these results. A second series of experiments, with still larger percentages of uranyl nitrate, show the retarding action still more plainly.

| $UO_2(NO_3)_2 + 6H_2O$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4066 gram. | 36·59 per cent. | 100·0 |
| 0·001 per cent. | 0·4000 | 36·00 | 98·3 |
| 0·002 | 0·3880 | 34·92 | 95·4 |
| 0·003 | 0·3034 | 27·30 | 74·6 |
| 0·004 | 0·2545 | 22·90 | 62·5 |
| 0·005 | 0·1550 | 13·99 | 38·2 |
| 0·008 | trace. | | |

The largest percentage of the salt used (0·008 per cent.), is seen to almost entirely prevent the action of the ferment, thus showing how extremely sensitive the salivary ferment is to the action of this salt. Comparing the two series of experiments, it is seen further, that a given percentage of the salt, say 0·001 per cent., is much more active in one case than in the other, indicating that the action of the salt is not constant. This is undoubtedly true to a limited extent. The action of a given percentage of the salt is constant only under *like conditions.* In the above series of experiments, the saliva is different in the two cases, and the real explanation of the difference in action is to be sought for in the amount of proteid matter contained in the saliva. Undoubtedly the retarding action of the uranium salt is checked, in part at least, like that of mercuric chloride,* by the

* Studies from this Laboratory, 1884–85, p. 71.

proteid matter of the saliva. Uranium is a well-known precipitant of albuminous matter and hence the larger the amount of albumin and globulin contained in the saliva, the weaker the retarding action of the uranium salt. Previous experiments have shown that saliva varies somewhat from day to day in its content of proteid matter, as well as in the amount of ferment, and although the former difficulty is obviated as much as possible by diluting the saliva, still this point must be borne in mind in making comparisons of the different series of experiments.

*Uranyl acetate.*

This salt appeared somewhat more inhibitory in its action than the nitrate, due possibly to its greater acidity. The two following series show the extent of action:

| $UO_2(C_2H_3O_2)_2 + H_2O$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4049 gram. | 36·44 per cent. | 100·0 |
| 0·001 per cent. | 0·2305 | 20·74 | 56·9 |
| 0·002 | 0·1698 | 15·28 | 41·9 |
| 0·003 | trace. | | |
| 0 | 0·4032 | 36·28 | 100·0 |
| 0·0003 | 0·4331 | 38·97 | 107·3 |
| 0·0005 | 0·3322 | 29·89 | 82·4 |
| 0·0008 | 0·3192 | 28·89 | 79·6 |
| 0·0010 | 0·2882 | 25·93 | 71·4 |

The presence of 0·003 per cent. of the salt almost entirely stops the action of the ferment, while 0·0003 per cent. decidedly increases amylolytic action. This latter influence is similar to that exerted by many other metallic salts in very small fractions of one per cent. and is doubtless to be attributed, in part at least, to the stimulating action of the acid-proteids formed, or in part, as suggested by Duggan,* to a more complete neutralization of the digestive fluid.

*Ammonio uranous sulphate.*

With this salt the following results were obtained:

| $U(SO_4)_2 + (NH_4)_2 SO_4 + H_2O$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3384 gram. | 30·45 per cent. | 100·0 |
| 0·0003 | 0·3935 | 35·41 | 116·3 |
| 0·0005 | 0·3798 | 34·18 | 112·2 |
| 0·0008 | 0·3563 | 30·40 | 99·8 |
| 0·001 | 0·3550 | 30·28 | 99·4 |
| 0·002 | 0·2951 | 26·55 | 87·2 |
| 0·003 | 0·0805 | 7·24 | 23·7 |

* See Amer. Chem. Jour. vol. viii, p. 211.

Here there is to be seen both stimulating and inhibitory action, both quite pronounced; and further, the salt is perfectly neutral, so that such action as is exerted must be due to the salt itself.

*Sodio uranic sulphate.*

This salt, which like the preceding was exactly neutral to test papers, shows both stimulating and retarding action, but in extent somewhat smaller than that of the uranous salt. Following are the results obtained:

| $UO_2SO_4 + Na_2SO_4$. | Total amount reducing bodies. | Starch converted | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4049 gram. | 36·44 per cent. | 100·0 |
| 0·0003 per cent. | 0·4100 | 36·90 | 101·3 |
| 0·0005 | 0·4000 | 36·00 | 98·8 |
| 0·0008 | 0·4100 | 36·90 | 101·2 |
| 0·001 | 0·4117 | 37·05 | 101·7 |
| 0·002 | 0·2530 | 22·77 | 62·5 |
| 0·003 | 0·2000 | 18·00 | 49·4 |
| 0·005 | trace. | | |

*Potassio uranic oxychloride.*

| $UO_2Cl_2 + 2KCl + 2H_2O$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4032 gram. | 36·26 per cent. | 100·0 |
| 0·0005 per cent. | 0·3951 | 35·55 | 98·0 |
| 0·0008 | 0·4016 | 36·14 | 99·6 |
| 0·001 | 0·4083 | 36·74 | 101·3 |
| 0·002 | 0·1881 | 16·92 | 46·6 |
| 0·003 | 0·1073 | 9·65 | 26·6 |
| 0·005 | trace | | |

This salt had an acid reaction and its retarding effects are seen to be somewhat more pronounced than that of the two preceding neutral salts.

*Ammonio uranic citrate.*

| $(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$. | Total amount reducing bodies | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4135 gram. | 37·21 per cent. | 100·0 |
| 0·0003 per cent. | 0·4298 | 38·68 | 103·9 |
| 0·0005 | 0·4016 | 36·14 | 97·1 |
| 0·0008 | 0·4049 | 36·44 | 97·9 |
| 0·001 | 0·3873 | 34·85 | 93·6 |
| 0·002 | 0·4016 | 36·14 | 97·1 |
| 0·003 | 0·3843 | 33·36 | 89·6 |

| $(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_2C_6H_5O_7$. | Total amount reducing bodies. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·4117 gram. | 37·05 per cent. | 100·0 |
| 0·004 per cent. | 0·2378 | 21·40 | 57·7 |
| 0·005 | 0·2144 | 19·29 | 52·0 |
| 0·006 | 0·2316 | 21·39 | 57·7 |
| 0·007 | 0·1845 | 16·60 | 44·8 |
| 0·008 | 0·1765 | 15·88 | 42·8 |
| 0·010 | trace | | |

The action of this salt is mainly a restraining one, but the action is pronounced only with the larger percentages.

As to the way in which these uranium salts diminish the amylolytic action of the ferment, we cannot say definitely. What has previously* been written regarding the action of other metallic salts, under like conditions, is doubtless true here. Loss of amylolytic power is due in part, no doubt, to partial direct destruction of the ferment, as well as to change in the reaction of the fluid. Coupled with this destructive action, however, there must be in addition something in the mere presence of these salts, dependent on chemical constitution, that controls the action of the ferment.

The following table shows the relative acceleration and retardation of the various salts, compared with their respective controls expressed as 100.

2. *Influence on the proteolytic action of pepsin-hydrochloric acid.*

The influence of uranium salts on the proteolytic action of pepsin-hydrochloric acid, was determined by ascertaining the amount of fibrin digested or dissolved in a given time, by a definite volume of a standard, artificial gastric juice, in the presence of varying amounts of the uranium salts. The gastric juice was made by dissolving 10 c.c. of a glycerin extract of pepsin in one litre of 0·2 per cent. hydrochloric acid. The volume of each digestive mixture was 50 c.c.; composed of 25 c.c. of the above mentioned artificial gastric juice and 25 c.c. of 0·2 per cent. hydrochloric acid, containing the necessary amount of uranium salt. The proteid material consisted of purified fibrin, coarsely powdered and dried at 100° C. One gram of fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for one hour and then the undissolved residue was collected on weighed filters and finally dried at 100–110° C. until of constant weight. The amount of fibrin dissolved is taken as a measure of the proteolytic action.

* See Studies from this Laboratory, vol. i, 1884–5, pp. 70–75.

TABLE SHOWING RELATIVE ACCELERATION AND RETARDATION OF AMYLOLYTIC ACTION.

| Percentage of Salts | 0·0001 | 0·0003 | 0·0005 | 0·0008 | 0·001 | 0·002 | 0·003 | 0·004 | 0·005 | 0·006 | 0·007 | 0·008 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Uranyl nitrate | 98·7 | 93·6 | 89·4 | ..... | 87·3 | ..... | 75·5 | 62·5 | 38·2 | ..... | ..... | 0 |
| Uranyl acetate | ..... | 107·3 | 82·4 | 79·6 | 71·4 | 41·9 | 0 | ..... | ..... | ..... | ..... | ..... |
| Ammonio uranous sulphate | ..... | 116·3 | 112·2 | 99·8 | 99·4 | 87·2 | 23·7 | ..... | ..... | ..... | ..... | ..... |
| Sodio uranic sulphate | ..... | 101·3 | 98·8 | 101·2 | 101·7 | 62·5 | 49·4 | ..... | 0 | ..... | ..... | ..... |
| Potassio uranic oxychloride | ..... | ..... | 98·0 | 99·6 | 101·3 | 46·6 | 26·6 | ..... | 0 | ..... | ..... | ..... |
| Ammonio uranic citrate | ..... | 103·9 | 97·1 | 97·9 | 93·6 | 97·1 | 89·6 | 57·7 | 52·0 | 57·7 | 44·8 | 42·8 |

Following are the results obtained with the various salts:

*Uranyl nitrate.*

| $UO_2(NO_3)_2 + 6H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1353 gram. | 86·47 per cent. | 100·0 |
| 0·025 per cent. | 0·1365 | 86·35 | 99·8 |
| 0·050 | 0·1378 | 86·22 | 99·7 |
| 0·100 | 0·2397 | 76·03 | 87·9 |
| 0·500 | 0·5003 | 49·97 | 57·8 |
| 1·000 | 0·6638 | 33·62 | 38·8 |

*Uranyl acetate.*

| $UO_2(C_2H_3O_2)_2 + H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1453 gram. | 85·47 per cent. | 100·0 |
| 0·025 per cent. | 0·1581 | 84·19 | 98·5 |
| 0·050 | 0·1867 | 81·33 | 95·1 |
| 0·100 | 0·2052 | 79·48 | 92·9 |
| 0·500 | 0·7507 | 24·93 | 29·2 |
| 1·000 | 1·0050 | 0 | 0 |

It is to be noticed here, that the retarding action of the acetate, as with saliva, is far greater than the nitrate, a fact which is doubtless dependent in this case on the nature of the acid united with the uranium. Further, it is to be noticed, that the action of uranyl sulphate falls about midway between the action of the nitrate and acetate. These facts accord with views previously* expressed, and show plainly that the extent of the retarding action of salts in general is dependent in part, on the liberation of the acid of the salt and the *digestive power* of the pepsin-*acid* formed. Experiments have shown that nitric acid of appropriate strength, united with pepsin, is about four-fifths as active as hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as hydrochloric of the same strength and that acetic acid is practically inactive. Hence, the base being the same, acetates, citrates, and other salts, the acids of which are not capable of working with pepsin will most readily retard gastric digestion. This view being correct, uranyl nitrate, sulphate and acetate should retard gastric digestion in just such relative proportion as our experiments show they actually do.

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* See Studies from this Laboratory, 1884–85, p. 94–95.

*Uranyl sulphate.*

| $UO_2SO_4 + 3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1832 gram. | 81·68 per cent. | 100·0 |
| 0·025 per cent. | 0·2545 | 74·55 | 91·3 |
| 0·050 | 0·2673 | 73·27 | 89·7 |
| 0·100 | 0·3155 | 68·45 | 83·8 |
| 0·500 | 0·6084 | 39·16 | 47·9 |
| 1·000 | 0·8225 | 17·75 | 21·7 |

*Ammonio uranous sulphate.*

| $USO_4 + (NH_4)_2SO_4 + H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1833 gram. | 81·67 per cent. | 100·0 |
| 0·025 per cent. | 0·2859 | 71·41 | 87·4 |
| 0·050 | 0·3143 | 68·57 | 83·9 |
| 0·100 | 0·3742 | 6·258 | 76·6 |
| 0·500 | 0·9113 | 8·87 | 10·8 |
| 1·000 | 1·0070 | 0 | 0 |

A comparison of the action of the two last salts, shows plainly that the ammonio uranous compound has a far greater inhibitory action than the simple uranyl sulphate.

*Ammonio uranic citrate.*

| $(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1747 gram. | 82·53 per cent. | 100·0 |
| 0·025 per cent. | 0·1795 | 82·05 | 99·4 |
| 0·050 | 0·2102 | 78·98 | 95·7 |
| 0·100 | 0·2180 | 78·20 | 94·7 |
| 0·500 | 0·9055 | 9·45 | 11·4 |
| 1·000 | 0 | 0 | 0 |

*Sodio uranic sulphate.*

| $UO_2SO_4 + Na_2SO_4 + 2H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2624 gram. | 73·76 per cent. | 100·0 |
| 0·025 per cent. | 0·2666 | 73·34 | 99·4 |
| 0·050 | 0·3688 | 63·12 | 85·5 |
| 0·100 | 0·4438 | 55·62 | 75·6 |
| 0·500 | 0·8131 | 19·69 | 26·7 |
| 1·000 | 0·9891 | 1·09 | 1·5 |

In the two last series, the ammonio uranic citrate is noticeable for not causing a gradual diminution in the proteolytic action of the ferment; but on the contrary, it gives rise to a sudden and rapid falling off in proteolytic action, when a certain percentage of the salt is added. The same thing is to be noticed in the case of the uranyl

acetate and the reason doubtless lies in the fact that the acid in these two salts is wholly incapable of forming an active compound with pepsin, and thus when a percentage of the salt is added sufficient to use up all of the hydrochloric acid of the gastric juice, digestive action comes to a full stop.

*Potassio uranic oxychloride.*

| $UO_2Cl_2 + 2KCl + 2H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3063 gram. | 69·37 per cent. | 100·0 |
| 0·025 per cent. | 0·2472 | 75·28 | 108·0 |
| 0·050 | 0·2123 | 78·77 | 113·5 |
| 0·100 | 0·2532 | 74·68 | 107·6 |
| 0·300 | 0·2648 | 73·52 | 105·9 |
| 0·500 | 0·3578 | 64·22 | 92·5 |

With this salt, unlike any of the preceding, there is to be seen a direct stimulating action on the ferment. Only in the presence of 0·5 per cent. of the salt is there any retarding effect produced. This naturally suggests that possibly uranium *per se*, at least in small fractions of a per cent., has really a stimulating effect on ferment action, but that owing to its combination with acids, in the formation of salts, its apparent effects in the case of pepsin-hydrochloric acid are those due to combination of the normal acid of the gastric juice and liberation of the acid of the uranium salt. In this way only, can we explain the noticeable difference in action of the oxychloride and the other uranium salts. Thus 0·5 per cent. of the former causes but slight diminution in proteolytic action, while with all the other salts, the same percentage causes on an average, a diminution in proteolytic action of at least 50 per cent. This would apply, of course, only to small percentages of uranium, for larger amounts of oxychloride would cause the formation of an indigestible uranium-albumin compound.

Here, however, as in the case of all the salts, the action of any given percentage is constant only under definite conditions. Diminish the amount of ferment, for example, and the amount of dissolved proteid matter consequent thereto, and then the retarding action of the same percentage of uranium salt will be correspondingly increased.

This is well illustrated in the following series of experiments with potassio uranic oxychloride. Using the same percentages of salt as employed in the preceding series, with the same strength of acid, but with only half the same content of pepsin extract, and the following results were obtained:

| Uranium salt. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·1652 gram. | 83·48 per cent. | 100·0 |
| 0·025 per cent. | 0·1982 | 80·18 | 96·0 |
| 0·050 | 0·2192 | 78·08 | 93·5 |
| 0·100 | 0·2569 | 74·31 | 89·2 |
| 0·300 | 0·3486 | 65·14 | 78·0 |

Increasing the percentages of the oxychloride still further, either with this strength of pepsin or the preceding, and there is seen a gradual diminution in the action of the ferment. Compared, however, with the action of the preceding salts, retardation is seen to be quite slow; thus even 2·0 per cent. causes a diminution of proteolytic action amounting to only 50 per cent.

The following table of comparisons shows the relative acceleration and retardation of the various salts compared with their respective controls expressed as 100.

TABLE SHOWING RELATIVE PROTEOLYTIC ACTION.

| Percentage of Salts | | 0·025 | 0·05 | 0·1 | 0·3 | 0·5 | 1·0 | 2.0 |
|---|---|---|---|---|---|---|---|---|
| Uranyl nitrate | | 99·8 | 99·7 | 87·9 | .... | 57·8 | 38·8 | .... |
| Uranyl acetate | | 98·5 | 95·1 | 92·9 | .... | 29.2 | 0 | ... |
| Uranyl sulphate | | 91·3 | 89·7 | 83·8 | .... | 47·9 | 2·17 | ... |
| Ammonio uranous sulphate | | 87·4 | 83·9 | 76·6 | .... | 10·8 | 0 | .... |
| Sodio uranic sulphate | | 99·4 | 85·5 | 75·6 | .... | 26·7 | 1·5 | ... |
| Ammonio uranic citrate | | 99·4 | 95·7 | 94·7 | .... | 11·4 | 0 | ... |
| Potassio uranic oxychloride | 1 | 108·0 | 113·5 | 107·6 | 105·9 | 92·5 | .. | .... |
| | 2 | 96·0 | 93·5 | 89·2 | 78·0 | 81·0 | 66·2 | 49·4 |

In retarding proteolytic action, the uranium salts act in part by combining with the proteid matter to be digested, forming a uranium-albumin compound, which is indigestible. Further, in a solution at all concentrated, the uranium salt is liable to precipitate mechanically a portion or all of the pepsin along with the albuminous matter. In addition to this, however, retardation is also due, as already expressed, to liberation of the acid of the salt by the hydrochloric acid of the gastric juice and to the subsequent formation of a pepsin-acid only partially, or not at all, capable of digestive action.

### 3. *Influence on the proteolytic action of trypsin.*

The method employed in determining the extent of proteolytic action in this case was much the same as in the preceding. The trypsin solution was made as neutral as possible and was prepared from dried ox pancreas, previously extracted with alcohol and ether; 20 grams dry pancreas, extracted with 200 c.c., 0·1 per cent. salicylic acid and ultimately diluted to 1 litre. A little thymol was added to prevent decomposition. 50 c.c. of the trypsin solution were used in each experiment, together with 1 gram of prepared fibrin and the necessary amount of uranium salt.

The first experiment was tried with uranyl nitrate, the mixtures being warmed at 40° C. for six hours. Following are the results:

| $UO_2(NO_3)_2 + 6H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·2927 gram. | 70·73 per cent. | 100·0 |
| 0·010 per cent. | 0·3328 | 66·72 | 94·3 |
| 0·025 | 0·3460 | 65·40 | 92·4 |
| 0·050 | 0·4198 | 58·02 | 82·0 |
| 0·100 | 0·5004 | 49·96 | 70·6 |
| 0·500 | —— | 0 | 0 |

With this salt retarding action is seen to be gradual up to a certain point, and then suddenly all ferment action ceases.

*Uranyl acetate.*

| $UO_2(C_2H_3O_2)_2 + H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4234 gram. | 57·66 per cent. | 100·0 |
| 0·010 per cent. | 0·4591 | 54·09 | 93·8 |
| 0·025 | 0·5460 | 45·40 | 78·7 |
| 0·050 | 0·6094 | 39·06 | 67·7 |
| 0·100 | 0·8173 | 18.27 | 31·6 |
| 0·500 | | 0 | 0 |

This series of experiments was warmed at 40° C. for about five hours. The inhibitory action of the salt is seen to be more pronounced than that of the nitrate; indeed, there is to be seen here, the same difference in action noticed in the case of the amylolytic ferment.

*Uranyl sulphate.*

With this salt, under exactly the same conditions of time and temperature as the preceding, the following results were obtained:

| $UO_2SO_4 + 3H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3790 gram. | 62·10 per cent. | 100·0 |
| 0·010 per cent. | 0·3863 | 61·37 | 98·8 |
| 0·025 | 0·5023 | 49·77 | 80·1 |
| 0·050 | 0·6066 | 39·34 | 63·3 |
| 0·100 | 0·8083 | 19·17 | 30·0 |
| 0·500 | —— | 0 | 0 |

These results are seen to accord almost exactly with the preceding and show that both salts have an action on this ferment much more pronounced than on pepsin-hydrochloric acid.

*Ammonio uranous sulphate.*

| $U(SO_4) + (NH_4)_2SO_4 + H_2O$ | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4025 gram. | 59·75 per cent. | 100·0 |
| 0·010 per cent. | 0·4031 | 59·69 | 99·7 |
| 0·025 | 0·4229 | 57·71 | 96·4 |
| 0·050 | 0·4824 | 51·76 | 86·6 |
| 0·100 | 0·5743 | 42·57 | 72·9 |
| 0·500 | —— | 0 | 0 |

*Sodio uranic sulphate.*

| $UO_2SO_4 + Na_2SO_4 + 2H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3261 gram. | 67·39 per cent. | 100·0 |
| 0·010 per cent. | 0·3414 | 65·86 | 97·4 |
| 0·025 | 0·3588 | 64·12 | 94·8 |
| 0·050 | 0·3961 | 60·39 | 89·5 |
| 0·100 | 0·4462 | 55·38 | 82·1 |
| 0·500 | —— | 0 | 0 |

*Ammonio uranic citrate.*

| $(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·3955 gram. | 60·45 per cent. | 100·0 |
| 0·010 per cent. | 0·4934 | 50·66 | 83·8 |
| 0·025 | 0·5282 | 47·18 | 78·0 |
| 0·050 | 0·6618 | 33·82 | 55·9 |
| 0·100 | 0·7705 | 22·95 | 37·8 |
| 0·500 | 0·7168 | 28·32 | 46·8 |

*Potassio uranic oxychloride.*

| $UO_2Cl_2 + 2KCl + 2H_2O$. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·4234 gram. | 56·76 per cent. | 100·0 |
| 0·010 per cent. | 0·4498 | 55·02 | 95·4 |
| 0·025 | 0·5224 | 47·76 | 82·8 |
| 0·050 | 9·5629 | 43·71 | 75·9 |
| 0·100 | 0·7087 | 29·13 | 50·8 |
| 0·500 | —— | 0 | 0 |

With the exception of ammonio uranic citrate, the four last salts experimented with, show about the same degree of inhibitory action; the citrate, however, appears less pronounced in its action than the others. As to the manner in which the uranium salts retard the proteolytic action of the pancreatic ferment, it is probable that the main explanation is to be found in the power possessed by the former of combining with proteid matter in general; combining with and rendering indigestible the albuminous material added to the digestive mixture and perhaps precipitating, or even destroying, the ferment itself. Further, the reason why certain salts appear less active than others is perhaps to be found in the fact that in the precipitation of albuminous matter by uranium salts, the uranium combines directly with the proteid matter, thus liberating the acid of the salt; and as trypsin is inactive in the presence of free mineral acids, and only partially active in the presence of combined acids (combined with proteids), it follows that an organic salt, such as a citrate, would naturally be less active as a retarding agent, than the nitrate or sulphate.

The following table shows the relative retardation of the various salts expressed in terms of relative proteolytic action:

TABLE SHOWING RELATIVE PROTEOLYTIC ACTION.

| Percentage of Salts | 0·01 | 0·025 | 0·05 | 0·1 | 0·5 |
|---|---|---|---|---|---|
| Uranyl nitrate | 94·3 | 92·4 | 82·0 | 70·6 | 0 |
| Uranyl acetate | 93·8 | 78·7 | 67.7 | 31·6 | 0 |
| Uranyl sulphate | 98·8 | 80·1 | 63·3 | 30·8 | 0 |
| Ammonio uranous sulphate | 99·7 | 96·4 | 86·6 | 72·9 | 0 |
| Sodio uranic sulphate | 97·4 | 94·8 | 89·5 | 82·1 | 0 |
| Ammonio uranic citrate | 83·8 | 78·0 | 55·9 | 37·8 | 46·8 |
| Potassio uranic oxychloride | 95·4 | 82·8 | 75·9 | 50·8 | 0 |

It is thus seen that uranium salts, in the main, like most other metallic salts, exert a decided retarding influence on the action of the digestive ferments.

## XVIII.—The Relative Distribution of Antimony in the organs and tissues of the body, under varying conditions.

By R. H. Chittenden and Joseph A. Blake, B.A., Ph.B.

Orfila, many years ago, proved that salts of antimony, like the salts of other metallic poisons, are absorbed and can be detected in the animal tissues and secretions, especially in the liver and kidneys; and further that the absorbed antimony is slowly discharged from these quarters through the medium of the urine. These early results were confirmed by other investigators, notably Danger and Flandin and by Panizza and Kramer, the latter of whom detected antimony, not only in the urine, but also in the blood of a man during a course of tartar emetic.* Orfila's work also indicated that while the elimination of absorbed antimony commences very quickly, it is a comparatively slow process; thus in one instance he stated † that he found antimony in the fat, liver and bones of a dog that had taken, three months and a half before its death, 46·5 grains of tartar emetic during a period of ten days, and that similar results were obtained in a second case in which the interval was four months. Presumably, however, elimination is much more rapid than these figures would seem to indicate.

Dr. Richardson, however, found antimony in abundant proportions in the liver, and in smaller proportions in the kidney and heart, twenty-one days after the last dose of antimony had been taken. As to the relative distribution of absorbed antimony, the experiments of Drs. Nevins and Richardson are the only important ones recorded. Dr. Nevins,‡ experimenting on rabbits, with tartar emetic in doses of 0·5, 1 and 2 grains four times daily, found that the weakest rabbit died after taking 12 grains, the strongest after taking 72 grains of the poison. Other rabbits were killed at varying lengths of time after taking the last dose of poison (31, 14, 4, and 3 days), and in every case antimony was found in large quantity in the liver, in smaller quantities in the spleen and stomach. Antimony was likewise found in the kidneys and urine of those animals that survived for some time, also in the lungs and in those that lived 15 days, in

* See Christison on Poisons, p. 372. † Traité de Toxicologie.

‡ See Reese, Manual of Toxicology, p. 259–260 and Woodman & Tidy, Forensic Medicine and Toxicology, p. 128–129.

the bones likewise. Dr. Nevins further states, that it was difficult to detect the poison in the muscles and in the blood, but it was found in the bones as late as the thirty-first day after discontinuing the poison.

Dr. Richardson* in 1856, examined the tissues of a dog that died 1 hour and 40 minutes after a solution containing a drachm of tartar emetic had been injected into the cellular tissue. The antimony was found in the following parts, in the order given as to quantity; blood, vomit, rectum, lungs, liver, stomach, bladder, kidneys and small intestines. In a second experiment, a wound in a dog's neck was dressed every morning with tartar emetic ointment, the dog dying at the end of the seventh day. In this case no antimony was found in the brain, but it was found in larger quantities in the liver and spleen than in the other organs. It is very evident, therefore, that tartar emetic, and presumably other salts of antimony likewise, will penetrate all the tissues of the body and that at the same time the antimony is constantly being eliminated by the kidneys. Further than this, the few results recorded indicate nothing definite. As Dr. Richardson well says, "the election of antimony by different parts of the body is as yet an open question; that the liver, however, would appear to be the structure in which it is most collected when the administration is slow and in small doses; and that the elimination of the poison is attempted by all the secreting surfaces." No positive statements can therefore be made regarding the relative distribution of antimony, other than in a general way.

Hence, it has been our object in the present investigation to study somewhat in detail, the relative distribution of antimony in the different tissues of the body under varying conditions; both as to the form of the poison and the manner of its introduction. As with arsenic, so with antimony, the relative proportion of poison found in the different tissues after death may become of considerable medico-legal importance, provided we have sufficient confirmatory data from which to draw conclusions. Particularly is it of importance to know the way in which the form of the poison will influence its distribution. Whether as with arsenic,† the administration of a soluble and diffusible form of the poison will lead to a noticeable accumulation in the brain or nerve tissue in general.

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*See Woodman & Tidy, p 129. Also Dr. W. B. Richardson, Abstract in Amer. Jour. Med Sciences, 1857, vol. 33, p. 266, and B. and F. Med. Chirurg. Rev. Oct 1856.

† See Amer. Chem. Jour., vol. v, p. 8, also Studies from the Laboratory of Physiological Chemistry, S.S.S. of Yale College, 1884–85, p. 141.

1. *The quantitative estimation of antimony.*

There are many methods by which antimony may be detected, even when present in quite small quantities; but there are only a few which yield accurate quantitative results, particularly in the presence of organic matter. In attempting to find a method sufficiently accurate for our purpose, we first tried the method recommended by Orfila, of introducing the final antimony solution into the Marsh apparatus; using for this purpose the form of apparatus and mode of procedure, found so efficacious in the case of arsenic.*. In every trial, however, there was a loss of at least 40 per cent. of the antimony; thus in the first case, with an amount of tartar emetic equivalent to 6 milligrams of metallic antimony, only 3·4 milligrams of the metal were recovered, and under exactly such conditions as with arsenic would lead to the recovery of the entire amount. And since in this trial experiment, the antimony was introduced directly into the Marsh apparatus, as tartar emetic dissolved simply in dilute sulphuric acid and without the presence of any organic matter to act as a hindrance, it follows that the loss must be due to retention of a portion of the antimony by the zinc and platinum. A second trial, with the same amount of antimony salt, gave a decided deposit of metallic antimony in the heated tube, but weighing only 3·5 milligrams. In this case the apparatus ran for three hours, but as before, it is evident that only a portion of the antimony was converted into antimoniuretted hydrogen, the remainder undoubtedly being retained either by the platinum used to alloy the zinc, or by the zinc itself. The presence of a small amount of platinum fused in with the zinc, previous to its granulation, does not appear to offer any obstacles to the complete evolution of arsenic as arseniuretted hydrogen. Numerous results obtained by one of us† testify to the accuracy of this statement. Bernstein had also noticed that alloying zinc with platinum or silver, did not hinder the complete evolution of the arsenic, while the addition of a little platinic chloride solution to the acid fluid from which arsenic was being evolved, led to the precipitation of even 50 per cent. of the arsenic present.‡ This probably is the explanation of the low results obtained by Hedden and Sadler in the estimation of arsenic by the Marsh apparatus, in the presence of platinum.§ But with antimony,

* See Amer. Chem. Jour., vol. ii., p. 235. † See Amer. Chem. Jour., vol. ii, p. 235.
‡ See Dragendorff, Gerichtlich chemische Mittheilung von Giften, p. 334, foot note.
§ See Amer. Chem. Jour., vol. vii, p. 342.

either the platinum alloyed with the zinc is sufficient to retain fully 50 per cent. of the metal, or as is very probable, the zinc itself causes a precipitation of a portion of the antimony. That this is a point overlooked by most writers on chemical toxicology, is evident from a perusal of the literature on the subject.

Whether it would be possible to obtain all of the antimony as antimoniuretted hydrogen, in the absence of any metal other than perfectly pure zinc, we cannot say. Certainly as a method for quantitative purposes, it would be too tedious a process to admit of general use, especially where such small amounts of antimony, as in our own experiments, would limit galvanic action to a minimum. That the method is capable of showing the presence of very small amounts of antimony, is unquestionable. Wormley's experiments* are very decided on this point, but evidently only a portion of the antimony will be recovered.

Precipitation of the antimony by hydrogen sulphide and final oxidation by fuming nitric acid and weighing as $Sb_2O_4$, gave fairly satisfactory results, when the weight of antimony was not less than 10 milligrams of $Sb_2O_4$. With smaller amounts, the results were far too high, owing probably to the far greater proportional increase of sulphur. Attempts to weigh as sulphide, after fusion of the first hydrogen sulphide precipitate with potassium nitrate and carbonate, likewise gave too high results when the amount of antimony was small. Further, the two latter methods are somewhat unsatisfactory, in that when the amount of antimony is very small, the nature of the final products is such, it is difficult to be certain of the purity of the matter weighed.

Owing to this reason partly, we next turned our attention to the electrolytic method for the separation and determination of antimony, as in this case the appearance of the metallic mirror is in itself a fair guarantee of the nature of the deposit, and its purity is easily proved.

Alex. Classen† has shown the accuracy of the method in general quantitative work, where moderate amounts of antimony are present (0·15—0·2 grams Sb). In his experiments, the antimony, in the form of sulphide, was dissolved in ammonium sulphide and the solution then exposed to electrolytic action. The ammonium sulphide solution must be free both from polysulphides and from free ammonia.

* Micro-Chemistry of Poisons, p. 229.

† Quantitative Analyse durch Elektrolyse. Berichte d. deutsch. chem. Gesell., xvii, p. 2474. See also Alex. Classen and Rob. Ludwig, ibid., xviii, p. 1104.

Classen also found that antimony was deposited quantitatively, when the sulphide was dissolved either in potassium or sodium monosulphide, or in potassium or sodium hydrosulphide. Polysulphides must also be absent in this case, and the potassium and sodium must be quite free from both iron and aluminum, as by long continued electrolytic action, sulphide of iron and aluminum hydroxide may be deposited upon the antimony. Classen also recommends the use of ammonium sulphydrate and a weak current; a strong current tending to cause the separation of the antimony in a pulverulent form, not closely adherent to the platinum.

As it would be necessary in our work, after oxidation of the organic matter, to separate the antimony as sulphide, the above method seemed particularly advantageous, and experiments were therefore tried to ascertain its value when applied to very small quantities. We employed a battery of four moderate sized gravity cells, giving a weak current, and as a rule, exposed the solution to the action of the current for at least 15 hours, as we found better results were obtained by the long continued action of a weak current, than by the quicker action of a more rapid one; particularly in such solutions as we usually had to work with, containing considerable excess of sulphur and some organic matter. The negative pole of the battery was either a small platinum crucible or a platinum dish, while the positive pole was a large piece of platinum foil welded onto a good sized platinum wire. The deposition of the antimony was much more complete, more tightly adherent to the platinum, and as a rule less mixed with sulphur under this arrangement, than when the dish was made to serve as the positive pole; due, doubtless, simply to the broader surface for the deposition of the metal.

Using ammonium sulphide as a solvent for the antimony sulphide, did not give us very good results by electrolysis, the loss being considerable. Much better results were obtained by using a solution of sodium monosulphide, made by saturating one-half of a 15 per cent. solution of sodium hydroxide with hydrogen sulphide, and then adding the remaining half of the sodium hydroxide solution.

The method was tested by precipitating definite volumes of a standard antimony (tartar emetic) solution with hydrogen sulphide, dissolving the metallic sulphide in the sodium monosulphide and then exposing the solutions to electrolytic action. When the separation of the metal was complete, it was found best to wash the deposit with considerable water, without breaking the current; as sometimes, as in the presence of tartaric acid, the separated metal rapidly dissolved

on discontinuing the current. The antimony was then finally washed with alcohol, any adherent sulphur lightly brushed off and the dish dried and weighed.

Following are some of the preliminary results obtained:

| Standard Sb Solution. | Theoretical Am't Sb. | Am't Sb found. | Duration of Electrolysis. |
|---|---|---|---|
| 50 c. c. | 0·0375 gram. | 0·0375 gram. | 16 hours. |
| 10 | 0·0075 | 0·0077 | 18 |
| 10 | 0·0075 | 0·0075 | 24 |
| 10 | 0·0075 | 0·0078 | 18 |
| 10 | 0·0075 | 0·0074 | 8 |
| 2 | 0·0015 | 0·0012 | 10 |
| 1 | 0·00075 | 0·0008 | 3 |

These results showed the method to be perfectly satisfactory for our purposes, and we, therefore, next tried the separation of small amounts of antimony from organic matter, and its final recovery by the above method.

As there was no doubt that large amounts of antimony could be satisfactorily recovered from organic matter, our experiments were confined mainly to very small quantities. In each experiment, 100 grams of either liver or beef were finely divided and to the so-prepared tissue, a few cubic centimetres of the standard antimony solution were added and the mixture *thoroughly* oxidized with hydrochloric acid and potassium chlorate. After removal of all free chlorine from the filtered fluid, by careful heating, the antimony was precipitated by hydrogen sulphide. This precipitate, which naturally contained, in addition to the sulphide of antimony, considerable sulphur and some organic matter, was then treated as follows: While still moist, after being freed from all hydrochloric acid by thorough washing, it was dissolved in the cold sodium monosulphide solution and then directly subjected to electrolysis. At first, we thought it necessary to free the precipitate from its excess of sulphur and organic matter by solution in ammonium sulphide, evaporation, fusion with potassium carbonate and nitrate, etc., obtaining it finally in the form of sulphide again, free from its former impurities. This, however, we found to be unnecessary; in fact the loss was far greater than the gain. Provided the oxidation with potassium chlorate be a thorough one and the free chlorine entirely removed from the solution, the first hydrogen sulphide precipitate is well adapted for electrolysis directly. It was found best, however, to keep the solution on the battery until all of the sulphur and organic matter was oxidized; that is until the reaction of the fluid had become acid. This took,

many times, 30 to 40 hours with our slow current. A more rapid current, would, to be sure, bring about a change in reaction much more quickly, but occasionally under such conditions the antimony would be less closely adherent and loss occur. Then again as the sulphur present was gradually changed into sulphuric acid, the final, strongly acid-reacting fluid, became a good conductor and so at the last, even with our four cells, electrolytic action was quite strong. In washing the deposited antimony, the acid fluid was syphoned out and water continuously added, without breaking the current, until the original fluid was entirely removed.

Following are a few of the results obtained, the antimony being added to 100 grams of tissue in each case.

| Standard Sb Solution. | Theoretical Am't Sb. | Am't Sb found. | Duration of electrolysis. |
|---|---|---|---|
| 10 c. c. | 0·0075 gram. | 0·0075 gram. | 24 hours. |
| 10 | 0·0075 | 0·0072 | 48 |
| 10 | 0·0075 | 0·0069 | 36 |
| 10 | 0·0075 | 0·0074 | 24 |
| 10 | 0·0075 | 0·0064 | 20 |
| 5 | 0·0037 | 0·0028 | 18 |

The results are certainly not all as close as those obtained in the absence of organic matter, but are perhaps as satisfactory as could be expected under the conditions of the experiment, viz: a large proportional amount of tissue (100 grams), a very small amount of metal and a large volume (say 500 c.c.) of fluid to precipitate from, with hydrogen sulphide. Even under the most unfavorable conditions, at least 75–80 per cent. of the antimony introduced into the organic matter was recovered.

We next turned our attention to the obtaining of some convenient and quick method for the direct determination of antimony in urine, or other like organic fluid. Preliminary experiments showed us that antimony, in the form of tartar emetic, could be separated completely from a tartaric acid solution by electrolysis. The separation takes place rapidly, but care must be taken to remove all of the tartaric acid solution by displacement with water, before breaking the current, otherwise the deposited antimony will instantly dissolve. Trial tests repeatedly gave results in close accord with theory. Excess of sodium tartrate, however, appeared to interfere somewhat with separation of the antimony; while the presence of sodium chloride in the presence of an excess of tartaric acid, prevented entirely the separation of the antimony. From a sulphuric acid solution, however, antimony was also deposited quantitatively, and on applying

this method to antimoniacal urine, we found it possible to recover the antimony without loss. The following results testify to the accuracy of the method:

In each experiment 25 c.c. of normal urine were employed, to which was added a number of cubic centimetres of the standard antimony solution and then 1 c.c. of pure dilute sulphuric acid, after which the solution was connected with the battery.

| Standard Sb Solution in 25 c. c. urine. | Theoretical amount Sb. | Amount Sb found. | Duration of electrolysis. |
|---|---|---|---|
| 10 c. c. | 0·0075 gram. | 0·0075 gram. | 48 hours. |
| 10 " | 0·0075 " | 0·0074 " | 24 " |
| 10 " | 0·0075 " | 0·0074 " | 20 " |
| 5 " | 0·00375 " | 0·0038 " | 18 " |
| 5 " | 0·00375 " | 0·0037 " | 18 " |
| 5 " | 0·00375 " | 0·0036 " | 15 " |
| 3 " | 0·00225 " | 0·0024 " | 10 " |
| 2 " | 0·0015 " | 0·0018 " | 20 " |
| 2 " | 0·0015 " | 0·0016 " | 18 " |
| 1 " | 0·00075 " | 0·0008 " | 16 " |

Doubtless all of these results could have been obtained equally as well in a very much shorter time, but most of the solutions were connected with the battery at night and allowed to run until morning, or whenever convenient. The method is evidently very accurate when applied in this manner, and we have made use of it very satisfactorily, even with 150 c. c. of urine, using in this case a platinum dish of 200 c. c. capacity as the negative electrode.

### 2. *Relative distribution of absorbed antimony.*

As already stated, the object sought in the following experiments was to ascertain the relative distribution of antimony under varying conditions; particularly, variations in the form of the poison, as its solubility or insolubility; in the method of introduction, as per mouth, rectum or sub-cutaneously; and lastly in the length of time during which the poison is being taken, whether in one large dose or in many small ones frequently repeated. All of these points we have endeavored to cover in the experiments about to be described.

#### Experiment I.

*Hypodermic injection of a solution of tartar emetic.*

0·120 gram of tartar emetic, dissolved in a little water, was introduced under the skin (right thigh) of a cat weighing 1262 grams.

3:10 p. m., solution injected.
3:16 " vomited copiously.
3:20 " " again, simply mucus.
3:23 " " mucus and bile.
3:38 " " and purged.
3:45 " partially paralyzed.
3:54 " vomited again.
4:30 " much prostrated.
5:10 " dead.

The various organs were then separated and the absorbed antimony determined, according to the method already indicated. Following are the results:

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Liver | 52·0 | 6·35 | 12·21 |
| Brain | 27·5 | 0·60 | 2·18 |
| Heart and lungs | 32·0 | 0·70 | 2·18 |
| Kidneys | 12·0 | 0·15 | 1·25 |
| Stomach and intestines | 74·0 | 0·80 | 1·08 |
| Muscle from back | 138·0 | 1·25 | 0·90 |
| | 335·5 | 9·85 | |

These figures show the greatest absorption by the liver; the brain stands next, while the muscle tissue appears to have absorbed but a relatively small amount of the antimony. In this connection it must be remembered that two hours only, intervened between the introduction of the poison and the death of the animal, hence it is evident that the brain tissue must have a decided tendency to hold absorbed antimony. The antimony, however, was introduced in the form of a readily soluble salt and under conditions directly favoring rapid and wide-spread distribution. That there is certainly some decided selective action, is evident from the fact that the muscle tissue, which must truly have had as good an opportunity as the brain tissue, retained per 100 grams of substance far less of the poison. Elimination had evidently commenced, for the kidneys contained a decided amount of antimony.

## Experiment II.

*Hypodermic injection of a solution of tartar emetic.*

In this experiment, a smaller amount of tartar emetic was used than in the preceding; and further, the poison was introduced in three distinct doses, thus allowing longer time for absorption. In fact, the animal lived 22 hours after the first dose, hence the experi-

ment stands in striking contrast to No. 1, in which the animal lived but two hours; while the results, contrasted with the preceding, show plainly the influence of time on the distribution of the poison. Following are the results of the experiment on a rabbit weighing 1295 grams.

| | | | | |
|---|---|---|---|---|
| Mar. 31, at 5:20 p.m., | injected under the skin of leg, | | 0·012 grm. | tartar emetic. |
| April 1, " 8:45 a.m., | " | " | 0·035 | " |
| April 1, "12:45 p.m., | " | " | 0·035 | " |
| | | Total, | 0·082 | |

Animal died at 3:05 p. m.

Following is the distribution of the antimony:

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Kidneys | 11·5 | 0·60 | 5·21 |
| Liver | 63·0 | 1·50 | 2·38 |
| Brain | 9·0 | 0·20 | 2·22 |
| Stomach and intestines | 98·0 | 2·00 | 2·04 |
| Heart and lungs | 17·0 | 0·25 | 1·47 |
| Muscle from back | 106·0 | 0·70 | 0·66 |
| | 304·5 | 5·25 | |

As might naturally be expected, the results indicate a more even distribution of the poison than in the preceding experiment. Although two-thirds as much antimony was used as in experiment No. 1, the liver contains a far smaller proportional amount of the poison than in the preceding experiment, while the kidneys stand first in their content of antimony. Between the brain and the liver, there is but little difference and the experiment plainly substantiates the preceding in showing the tendency of brain tissue, under these conditions, to absorb and retain antimony. In the muscle tissue the percentage of absorbed antimony is almost exactly the same as in No. 1, that is, proportional to the amount of antimony introduced. The animal had evidently lived long enough to admit of a fairly complete distribution of the poison, and elimination having been going on for some time, those parts which had originally contained the most, particularly the liver, had been drawn on to the greatest extent; so that at the time death intervened, the excretory organs, notably the kidneys, were the richest in poison. This fact further indicates that the elimination of absorbed antimony proceeds somewhat rapidly.

### Experiment III.

*Hypodermic injection of a solution of tartar emetic.*

In this experiment, a cat weighing 1613 grams, had injected under the skin of its hind leg 0·150 gram of tartar emetic in one dose. There was some purging and vomiting, and the animal died in 4¼ hours after the administration of the poison. The object sought in this experiment, which is virtually a repetition of No. 1, with a somewhat larger dose of antimony, was simply to see whether there would be found the same relative absorption of antimony by the liver and kidney as in No. 1, and if by chance there should occur a longer interval of time between the introduction of the poison and death, what then would be the relative amounts of antimony in the two organs. As stated above, the animal lived 4¼ hours after the administration of the poison, or 2¼ longer than the cat in No. 1. Following are the results of the analysis of the two organs:

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Liver | 62·0 | 2·50 | 4·03 |
| Kidneys | 14·5 | 0·25 | 1·72 |

These confirm to a certain extent the results of No. 1, while at the same time the smaller difference between the amount of antimony contained in the liver and kidneys, as compared with the difference found at the end of two hours (see experiment I), would seem to indicate that the liver had already absorbed its maximum amount, and that at the time of death, elimination was well under way; or in other words, that the removal of the absorbed antimony from the liver had already commenced.

### Experiment IV.

(*a.*) *Hypodermic injection of a solution of tartar emetic.*
(*b.*) *Injection of a solution of tartar emetic per rectum.*

These two experiments were undertaken to ascertain whether the avenue by which the poison was introduced, would influence materially the relative absorption of the antimony. The results, however, although interesting, do not definitely answer the question. Absorption by injection per rectum is so much slower than by hypodermic injection, or the effects produced are so much slower in manifesting themselves, that it is impossible to have the conditions exactly alike in the two cases. Either the time required to produce a given

effect, in the case of injection per rectum, will be longer than by hypodermic injection, or else the amount of poison must be correspondingly increased; either of which introduces an objectionable element into the experiment.

(*a.*) Rabbit weighing 1485 grams had injected under its skin 0·80 gram of tartar emetic dissolved in a little water. Injection made at 11:35 a. m. At 3:45 p. m., 4 hours and 10 minutes after the first injection, 0·08 gram more was injected in the same manner. At 4:05 p. m. the animal died.

(*b.*) Rabbit weighing 1512 grams had injected per rectum 0·08 gram of tartar emetic dissolved in a little water. Injection made at 11:45 a. m. At 3:50 p. m. the animal apparently not being affected at all, whereas rabbit (*a*) was strongly under the influence of the poison, 0·160 gram more of the salt was injected per rectum as before. At 5:30 p. m. the animal was still alive, but evidently feeling the effect of the poison. The animal died during the night.

Following are the results of the analysis of the parts from the two rabbits:

RABBIT (*a*) *hypodermic injection.*

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tisssue, milligrams. |
|---|---|---|---|
| Kidneys | 10·2 | 0·65 | 6·34 |
| Brain | 5·5 | 0·20 | 3·63 |
| Liver | 54·0 | 1·30 | 2·40 |
| Heart and lungs | 15·5 | 0·30 | 1·93 |
| Stomach and intestines | 174·0 | 1·60 | 0·92 |
| Muscle | 110·0 | 0·50 | 0·45 |
| | 369·2 | 4·55 | |

RABBIT (*b*) *injection per rectum.*

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Stomach and small intestines | 172 | 8·89 | 15·30 |
| Brain | 9 | 0·40 | 4·40 |
| Rectum and adjoining intestine | 18 | 0·55 | 3·05 |
| Liver | 54 | 1·60 | 2·96 |
| Kidneys | 13 | 0·25 | 1·92 |
| Muscle | 100 | 1·10 | 1·11 |
| Urine | 20 | 0·20 | 1·10 |
| Heart and lungs | 17 | *trace* | |
| | 403 | 12·99 | |

Comparing first, the results obtained from rabbit (*a*) with those of the three preceding experiments, we see at once that the distribution of the antimony is much the same as in No. 2, in which, however, the animal lived nearly 22 hours after the introduction of the first dose of poison and somewhat over two hours after the last. The fatal dose, moreover, in this case was nearly the same in amount as the first dose in experiment IV *a*.

The conditions, however, of this experiment (IV *a*) do not exactly accord with any of the preceding, hence close comparisons cannot well be made. The brain, as in all of the experiments with tartar emetic, contains a proportionally large amount of antimony, while the muscle contains a very small amount. The only thing in this experiment not exactly in accord with the preceding experiments, is the proportionally large amount of poison in the kidneys, as compared with the liver. The only apparent explanation seems to be that, the first dose being small, the liver had quickly reached its maximum absorption and elimination had been rapidly going on; so that at the end of the four hours intervening between the first and second doses of the poison, the kidneys had drawn extensively from the liver, rapidly diminishing its content of the poison. Further, after the second dose of poison, the time before death was so short that the additional absorption by the liver was not sufficient to make up the deficiency, and hence the results found. In this connection, it must be remembered that tartar emetic is very readily soluble and diffusible, and that being injected in solution directly under the skin, its absorption must necessarily be very complete and rapid.

In Rabbit (*b*) the conditions are wholly different from those of the preceding experiments. In all, 0·24 gram of tartar emetic, dissolved in water was introduced into the rectum and 8–10 hours, at least, must have intervened between the administration of the first dose of the poison and death. That the stomach and small intestines should contain the largest proportional amount of antimony is perhaps not at all strange, since the antimony solution would naturally pass rapidly by osmosis through the entire alimentary tract. That this, however, is not the full explanation, is evident, when we compare the amount found in the large intestine with the former. If due simply to osmosis, the percentage amount of antimony would be about the same all through the intestines; hence we must look to some selective action for explanation of the increased amount found in the small intestines. In all of the preceding experiments, the amount of antimony found in the stomach and intestines has been

considerably greater than in the muscle tissue. Undoubtedly, the greater vascularity of the former has much to do with its greater content of the metal, but even this is not sufficient to account for all of the antimony found; for whenever blood itself has been analyzed, the amount of antimony has not been large. Unquestionably then, we must assume special absorptive action on the part of the epithelial cells of the stomach and small intestines. In this connection it is well to notice the work of Brinton, who proved that when tartar emetic was injected into the vein of an animal, it was very freely and rapidly eliminated by the stomach. This was also corroborated by Dr. Richardson who, in addition, found that a similar elimination followed the inhalation of antimoniuretted hydrogen.* In addition, it may be that absorption of antimony from the alimentary tract goes on slowly and that hence only a portion was removed. This idea has considerable to support it, when we consider the distribution of the absorbed antimony. Remembering that in this experiment, a larger amount of antimony was used than in any of the preceding ones, and that there was apparently ample time for absorption, one cannot help but think that the content of antimony in the remaining tissues and organs is very small. This is very evident, and must be due to one of two causes; either there has been a lack of absorption or else elimination has been going on very rapidly. The brain contains a noticeable amount of antimony, larger than found in any preceding case, while the liver and kidneys both contain a comparatively small amount. The amount of antimony in the kidneys and particularly the amount in the urine, plainly indicates that elimination was going on rapidly; but the fact that the percentage content of antimony in the liver is greater than in the kidneys, would perhaps indicate that at the time of death, absorption was not completed. Such being the case, the only inference to be drawn from the two preceding experiments, is that the introduction of tartar emetic into the rectum leads simply to a much slower absorption and distribution of the antimony than by hypodermic injection, but that there is no essential difference in the relative distribution of the poison under these two conditions.

* Quoted by H. C. Wood, Therapeutics, p. 159.

### Experiment V.

(*a.*) *Tartar emetic in substance, introduced into the stomach.*

(*b.*) *Antimonious oxide* ($Sb_2O_3$) *introduced into the stomach.*

In this experiment there were two objects in view; one was to see the effect of tartar emetic in substance, as compared with the action of the same salt introduced into the system by hypodermic injection or per rectum; the second, to compare the absorption of an *insoluble* compound of antimony ($Sb_2O_3$) with that of the more soluble tartrate.

In this experiment two dogs were used and the poison was fed to them at regular intervals, in small doses, for a period, in each case, of 17 days. The animals were then killed and the various parts analyzed. The two experiments were exactly alike in every respect, except in the amount of poison administered.

(*a.*) Dog weighing 7·75 kilos was fed 0·762 gram of tartar emetic during a period of 17 days, in two or three doses daily, the individual doses being small enough not to induce vomiting. The first two days, the dose was 0·016 gram per day, the third 0·020 gram, the fourth 0·030 gram and so on, increasing each day until the last daily dose was 0·085 gram of the poison. The dog was then killed by chloroform, just six hours after the last dose of poison was administered.

(*b.*) Dog weighing 14·2 kilos was fed 2·073 grams of antimonious oxide, during a period of 17 days, in two daily doses of from 0·032 to 0·125 gram per day. The dog was then killed by chloroform, 18 hours after the last dose of antimony was given.

Following are the results of the analysis of the various parts:

Dog (*a*) *with tartar emetic*—(0·762 gram).

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Liver | 304 | 17·80 | 5·85 |
| Salivary glands | 11 | 0·25 | 2·27 |
| Kidneys | 58 | 1·25 | 2·15 |
| Brain | 76 | 1·15 | 1·51 |
| Tongue | 36 | 0·40 | 1·11 |
| Muscle (thigh) | 150 | 1·60 | 1·06 |
| Spleen | 19 | 0·15 | 0·80 |
| Heart | 77 | 0·50 | 0·66 |
| Lung | 140 | 0·50 | 0·36 |
| Bone (femur and tibia) | 43 | 0·10 | 0·23 |
| Blood | 130 | 0·20 | 0·15 |
| Testes | 12 | *trace* | |
| Pancreas | 23 | *trace* | |
| | 1079 | 24·05 | |

Dog (*b*) *with antimonious oxide*—(2·073 grams).

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Liver | 452 | 23·70 | 5·24 |
| Lungs | 140 | 1·80 | 1·28 |
| Muscle (fore leg) | 157 | 1·20 | 0·76 |
| Brain | 79 | 0·40 | 0·50 |
| Muscle (thigh) | 200 | 0·90 | 0·45 |
| Kidneys | 82 | 0·10 | 0·12 |
| Heart | 117 | *trace* | |
| Blood | 440 | *trace* | |
| | 1667 | 28·10 | |

In considering these results, we notice first that in dog (*a*) the distribution of the poison is much the same as in the preceding experiments with tartar emetic, viz: the liver, kidneys and brain stand first in their content of antimony. That the liver should contain more per 100 grams than the kidneys, although the animal lived full eight hours after the last dose of poison was taken, is here to be expected, since absorption as a whole would naturally be slower than in some of the preceding experiments; and, further, in this case probably all of the antimony would be absorbed through the portal circulation. In the case of dog (*b*), the conditions are different from any heretofore; we have here an insoluble form of antimony contrasted with a readily soluble and diffusible salt. Solution must necessarily be somewhat slow in this case, but the acid juices of the stomach unquestionably do dissolve and render diffusible, at least a portion of, this form of the poison.

We notice first that the total amount of antimony administered, is fully three times as much as the amount of tartar emetic given, and yet the amount of antimony recovered from the different tissues and organs is but 4 milligrams more than in the case of tartar emetic. This suggests that either considerable antimony is excreted by the kidneys (more than in the case of tartar emetic) or else that considerable passes through the alimentary tract unabsorbed. The dog being confined in a cage of suitable construction, the 24 hours' urine was collected on several occasions and the amount of antimony determined. Thus on one day, when 0·097 gram of antimonious oxide had been administered, following after a daily dose of 0·064 gram, the 24 hours' urine contained 13·5 milligrams of antimony (Sb). Later, at a time when the daily dose was 0·130 gram of the oxide, the 24 hours' urine contained 22·5 milligrams of antimony. Hence it is plain that

considerable of the antimony given was being absorbed; but bearing in mind that this latter amount was the largest excreted by the kidneys in any one day, and further that the daily dose of antimony was being increased each day rather than diminished, it is also plainly evident from the amount of absorbed antimony found, that a certain portion must pass through the alimentary canal unabsorbed. Further, the small amount of antimony found in the kidneys supplements this view, as does also the noticeably small amount of absorbed antimony found throughout the body, aside from the liver.

One of the main objects in trying this last experiment was to see what influence the form of the poison would have on its absorption by the brain. With arsenic, it has been plainly demonstrated by one of us,* as well as by other workers in this field, that soluble and readily diffusible forms of arsenic are absorbed by the brain in appreciable quantities, while arsenious oxide for example, no matter whether taken in large or small doses, single or oft-repeated, is never found in the brain other than in mere traces. With antimony we had expected to see something of the same kind. The results, however, although tending in that direction, are not quite as decisive as we should have liked. The antimony found in the brain in the antimonious oxide case is, to be sure, considerably smaller in amount than that found in (*a*), although the dose of antimony given in the former was much larger than in the latter case. But it is also to be seen in the antimonious oxide case, that the amount of absorbed antimony in the brain, although very small, is still greater than the amount found in the kidneys or muscle.

We attempted another experiment in the same direction with rabbits, but as the amounts of antimony found in the brain in both animals were hardly more than mere traces, the results do not give us any additional light on the matter. In spite of the fact that the experiment was a failure, so far as its main object was concerned, we venture to describe it, since it well illustrates in other respects, the greater virulence and diffusibility of tartar emetic. Two rabbits of nearly equal weight were selected, and to one potassium antimony tartrate was fed in gradually increasing doses for a period of 17 days, at the end of which time the animal died with all the symptoms of antimoniacal poisoning. To the other rabbit, antimonious oxide was fed for the same period of time, in doses the same as given to the first rabbit; that is, doses equivalent to the antimony (Sb) contained in the tartar emetic. Each rabbit, therefore, received

* See Studies from this Laboratory, vol. i, for the year 1884–85, p. 141.

twice a day, the same equivalent of antimony, and at the end of the 17 days the one rabbit had taken 2·34 grams of tartar emetic, the other 1·08 grams of antimonious oxide. While each rabbit had taken the same amount of antimony, the one which had taken it in the form of potassium antimony tartrate was much more severely affected by the poison. In this case there was severe purging and finally death on the 17th day. In the case of the rabbit fed with antimonious oxide, the only apparent effect of the poison was a loss of appetite and great thirst. This animal was killed with chloroform on the death of the first rabbit. Both forms of antimony were administered as powders, by way of the mouth, in small gelatin capsules.

Following are the results of the analysis of the various parts from the two rabbits:

RABBIT (*a*) *fed with tartar emetic.*

| | Total weight, grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Liver | 50·0 | 4·8 | 9·60 |
| Kidneys | 6·7 | 0·5 | 7·40 |
| Heart and lungs | 18·0 | 0·4 | 2·22 |
| Muscle from back | 55·0 | 0·5 | 0·91 |
| Muscle from legs | 79·0 | 0·3 | 0·38 |
| Brain | 7·7 | *trace* | |
| | 216·4 | 6·5 | |

RABBIT (*b*) *fed with antimonious oxide.*

| | Total weight. grams. | Weight of Sb, milligrams. | Sb per 100 grams of tissue, milligrams. |
|---|---|---|---|
| Liver | 57·0 | 1·3 | 2·28 |
| Muscle from back | 77·0 | 0·7 | 0·90 |
| Muscle from legs | 100·0 | 0·7 | 0·70 |
| Kidneys | 8·0 | *trace* | |
| Heart and lungs | 16·0 | *trace* | |
| Brain | 8·5 | *trace* | |
| | 266·5 | 2·7 | |

Looking at these results and remembering that each animal received the same amount of metallic antimony, it is evident that tartar emetic is much more completely absorbed than the oxide. With tartar emetic, however, the results are not exactly in accord with the previous ones, obtained with this salt; thus the amount of antimony absorbed by the brain is far smaller proportionally than

found hitherto. To be sure, the compound was not in the previous experiments introduced into the stomach of a rabbit in the form of powder, and it is possible that the reason for the difference in the amount of antimony found in the brain in this case and that in the brain of the dog similarly treated, lies in the fact of a slower absorption from the stomach of a herbivorous animal.

## XIX.—Influence of Antimonious Oxide on Metabolism. By R. H. Chittenden and Joseph A. Blake.

The physiological action of antimony has been studied mainly with potassium antimony tartrate, the form in which antimony is most commonly used therapeutically. No experiments, however, appear to have been made, even with this salt, to ascertain the influence of antimony on the metabolism of the body. Gäthgens, however, as quoted by Dr. H. C. Wood,* found in some incomplete experiments an increase in the elimination of urea after repeated non-toxic doses of antimony. It is further reported† that antimonic acid or other preparations of the metal, when taken in half gram doses daily for about two weeks, cause a diminution in the amount of glycogen in the liver and even a total disappearance of it, and that the liver, kidneys and heart undergo fatty degeneration. Grohe and Mosler‡ have confirmed the latter and state that in the production of the famous fatty livers, a certain amount of the white oxide of antimony is fed to the geese daily. Aside from these facts, there appears little definite regarding the action of antimony on the physiology of nutrition.

What we have, therefore, endeavored to ascertain in the present experiment is the influence of antimony on metabolism; or particularly, on proteid metabolism as manifested in the excretion of nitrogen, sulphur and phosphorus. Previous experiments§ have shown that potassium antimony tartrate has a noticeable retarding action on pancreatic digestion; we have not, however, deemed it best in the present experiments to use tartar emetic, as the ready solubility and diffusibility of the compound might cause too rapid absorption and thus lead to speedy toxic action. In spite, therefore, of the fact that we have not made any experiments on the influence of antimonious oxide on digestive action, we have preferred to use the latter in the present experiments, because of its probable slower toxic action and also because it has been so extensively used as a means to induce, or to aid in the production of, fatty degeneration.

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* Therapeutics, Materia Medica and Toxicology, p. 156.

† See Virchow's Archiv., 1865, Band xxxiv, p. 78.

‡ Compare H. C. Wood. Therapeutics, p. 161.

§ Studies from this Laboratory, 1884–85, p. 105.

Our experiments were made on a setter dog, weighing 12·6 kilos. The animal was confined in a suitable cage, so that the excretions could be collected daily without loss. The food consisted of fresh beef and crackers, together with a suitable amount of water. The beef was prepared as follows: About 40 lbs. of fresh beef, freed from fat, tendons, etc., was finely divided by passing through a sausage machine and then dried at a low temperature until it had lost about 75 per cent. of water, and was in a condition suitable for preservation. 50 grams of this preserved meat, together with 75 grams of the sampled crackers, soaked in 300 c.c. of water, were fed to the dog twice daily. The meat, as determined by Kjeldahl's method, contained 12·4 per cent. of nitrogen, while the crackers contained 1·9 per cent. Hence the dog was fed daily 15·25 grams of nitrogen.

On May 11th, the dog was put upon this diet and from the 17th on, the 24 hours' urine was collected daily and analyzed. After a period of two weeks, during which daily analysis of the urine had shown a fairly constant composition, antimonious oxide was added to the diet in the quantities indicated in the table of results; the diet of course continuing the same throughout the length of the experiment.

We deemed it better, as well as more accurate, to measure the influence of the antimony by a daily determination of the total nitrogen, sulphur and phosphorus of the urine, rather than to attempt a determination of urea, uric acid, phosphoric acid, etc. Nitrogen, we determined, according to the method of Kjeldahl,* modified slightly as suggested by Dr. E. H. Jenkins, of the Agricultural Experiment Station, viz: 5 c.c. of the acid urine were placed in a long pear-shaped bulb and evaporated to dryness quickly on a water bath. The residue was heated directly over a small flame with 10 c.c. of pure concentrated sulphuric acid and 0·7 gram of oxide of mercury, until oxidation was almost complete. Then, a little finely powdered potassium permanganate was added, to render the oxidation quite complete. The solution was then diluted, an equivalent amount of potassium sulphide added to convert the mercury into sulphide, and lastly a standard solution of sodium hydroxide, after which the ammonia was driven off by boiling and collected in standard acid.

Total phosphorus and sulphur were determined as follows: 50 c.c. of urine were evaporated in a capacious silver dish with 10 grams of potassium hydroxide and 10 grams of potassium nitrate and the resi-

* Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Zeitschrift für analytische chemie, xxii, 366.

due heated carefully until the organic matter was completely oxidized. The fused mass was then dissolved in water and diluted to 250 c.c. Of this, 100 c.c., representing 20 c.c. of the original urine, were used for the sulphur, while the second 100 c.c. were used for the phosphorous, determination. For sulphur, the 100 c.c. were acidified with hydrochloric acid and evaporated to dryness on a water bath in order to remove all nitrate and nitrite. The residue was then dissolved in water acidified with hydrochloric acid, and the sulphuric acid precipitated with barium chloride in the usual manner. For phosphorus, the 100 c.c. were acidified with nitric acid, evaporated to dryness, the residue dissolved in water, acidified with nitric acid and the phosphoric acid precipitated with molybdenum solution. This precipitate was then dissolved in a dilute solution of ammonia, the phosphoric acid reprecipitated as ammonio-magnesium phosphate, and the phosphorus finally weighed as magnesium pyrophosphate. Chlorine was determined volumetrically in the usual manner, with a standard solution of silver nitrate, after destruction of the organic matter by fusion with potassium nitrate, etc.

The results, expressed in grams per 24 hours, are shown in the accompanying tables. The 24 hours' urine represents the quantity passed from 9 A. M. of one day to 9 A. M. of the next. As, however, the animal was not always regular in its passage of urine, it frequently happened that the quantity on one day would be very small, while on the next it would be correspondingly increased, without any change in specific gravity, and with a daily average corresponding to the normal, as for example on May 25th and 26th.

In order, therefore, to obviate the difficulty which this irregularity tends to introduce into the results, we have added to the table a daily average of each three days results; a study of which shows plainly that antimonious oxide, in the present experiment at least, does not have any noticeable influence on the excretion of any of the elements determined. Numerically, there is a slight increase in the amount of nitrogen excreted during the taking of the antimony, but the increase is noticeable only in the grand average and is altogether too small to be of much significance. Further, it is to be noticed that the average for the two series does not show any corresponding increase in sulphur. If antimony causes an increased excretion of nitrogen, it means an increase in proteid metabolism, which should in turn give rise to an increased excretion of sulphur and phosphorus. It is to be noticed in the daily results, that the excretion of sulphur nd phosphorus runs parallel with the excretion of nitrogen; an in-

WITHOUT ANTIMONY.

| Date. | Reaction. | Volume. | Sp. Gr. | Nitrogen. | Phosphorus. | Sulphur. | Chlorine. | Amount of $Sb_2O_3$ taken. |
|---|---|---|---|---|---|---|---|---|
| | | c. c. | | grams. | gram. | gram. | gram. | grains. |
| May 17. | acid. | 462 | 1024·5 | 10·526 | 0·6122 | 0·5789 | 0.7239 | |
| 18. | acid. | 510 | 1024·5 | 10·949 | 0·6587 | 0·6289 | 0·7203 | |
| 19. | acid. | 469 | 1027·5 | 11·898 | 0·7355 | 0·6716 | 0·5871 | |
| Daily average..... | | *480* | *1025·5* | *11·124* | *0·6688* | *0·6264* | *0·6771* | |
| May 20. | alkaline. | 470 | 1023·5 | 9·638 | 0·5716 | 0·5606 | 0·6720 | |
| 21. | acid. | 466 | 1027·0 | 11·904 | 0·7118 | 0·6953 | 0·3792 | |
| *23. | acid. | 468 | 1029·5 | 13·226 | 0·7646 | 0·7738 | 0·5126 | |
| Daily average..... | | *468* | *1026·6* | *11·589* | *0·6826* | *0·6765* | *0·5212* | |
| May 24. | acid. | 406 | 1030 0 | 11·492 | 0·6536 | 0·6654 | 0·4574 | |
| 25. | acid. | 392 | 1027·0 | 9·512 | 0·5599 | 0·6193 | 0·7852 | |
| 26. | acid. | 648 | 1027·5 | 16·294 | 1·2396 | 0·9709 | 0·9329 | |

WITHOUT ANTIMONY—*continued.*

| Date. | Reaction. | Volume. | Sp. Gr. | Nitrogen. | Phosphorus. | Sulphur. | Chlorine. | Amount of $Sb_2O_3$ taken. |
|---|---|---|---|---|---|---|---|---|
| | | c. c. | | grams. | gram. | gram. | gram. | grains. |
| May 27. | acid. | 430 | 1029·5 | 11·638 | 0·7379 | 0·6888 | 0·6191 | |
| 28. | acid. | 414 | 1028·0 | 10·593 | 0·6179 | 0·6243 | 0·5422 | |
| 29. | acid. | 455 | 1028·0 | 11·404 | 0·7421 | 0·6527 | 0·5411 | |
| Daily average | | *433* | *1028·5* | *11·211* | *0·6993* | *0·6552* | *0·5674* | |
| June† 3. | acid. | 500 | 1027·5 | 13·254 | 0·8469 | 0·6860 | 0·1878 | |
| 4. | acid. | 482 | 1027·5 | 12·724 | 0·7753 | 0·6593 | 0·3922 | |
| 5. | acid. | 414 | 1028·5 | 11·102 | 0·6503 | 0·5884 | 0·3369 | 0·5<br>0·5 |
| Daily average | | *455* | *1027·8* | *12·360* | *0·7575* | *0·6445* | *0·3056* | |
| Average for the series | | *466* | *1027·3* | *11·743* | *0·7251* | *0·6708* | *0·5592* | |

† Between the 29th of May and the 3d of June the urine was not analyzed, as the dog seemed somewhat unwell and in poor appetite.

WITH ANTIMONIOUS OXIDE.

| Date. | Reaction. | Volume. | Sp. Gr. | Nitrogen. | Phosphorus. | Sulphur. | Chlorine. | Amount of $Sb_2O_3$ taken. |
|---|---|---|---|---|---|---|---|---|
| | | c. c. | | grams. | gram. | gram. | gram. | grains. |
| June 6. | acid. | 490 | 1030·0 | 12·968 | 0·9011 | 0·6743 | 0·6521 | 0·5, 0·5 |
| 7. | acid. | 350 | 1030·0 | 10·060 | 0·5961 | 0·5018 | 0·4601 | 0·5, 0·5 |
| 8. | acid. | 560 | 1026·5 | 13·226 | 0·8146 | 0·7780 | 0·6361 | 0·5, 0·5 |
| Daily average..... | | *466* | *1028·8* | *12·084* | *0·7706* | *0·6513* | *0·5827* | |
| June 9. | acid. | 432 | 1030·0 | 12·587 | 0·7576 | 0·6333 | 0·4597 | 0·5, 1·0 |
| 10. | acid. | 474 | 1027·5 | 11·838 | 0·7068 | 0·6275 | 0·6898 | 0·5, 0·5 |
| 11. | acid. | 424 | 1028·0 | 11·890 | 0·7131 | 0·5977 | 0·3450 | 1·0, 0·5 |
| Daily average..... | | *443* | *1028·5* | *12·105* | *0·7258* | *0·6161* | *0·4931* | |

WITH ANTIMONIOUS OXIDE—*continued.*

| Date. | Reaction. | Volume. | Sp. Gr. | Nitrogen. | Phosphorus. | Sulphur. | Chlorine. | Amount of $Sb_2O_3$ taken. |
|---|---|---|---|---|---|---|---|---|
| | | c. c. | | grams. | gram. | gram. | gram. | grains. |
| June 12. | acid. | 504 | 1030·0 | 13·648 | 0·8550 | 0·7212 | 0·8586 | 0·5<br>1·0 |
| 13. | acid. | 416 | 1027·0 | 10·645 | 0·6320 | 0·5307 | 0·5972 | 0·5<br>0·5 |
| 14. | alkaline. | 478 | 1028·5 | 12·900 | 0·7490 | 0·8381 | 0·8571 | 1·0<br>0·5 |
| Daily average..... | | *466* | *1028·5* | *12·397* | *0·7453* | *0·6966* | *0·4359* | |
| June 15. | acid. | 404 | 1030·5 | 11·471 | 0·6802 | 0·6788 | 0·5568 | 1·0<br>1·0 |
| 16. | acid. | 484 | 1028·0 | 12·173 | 0·7664 | 0·6414 | 0·5453 | 1·0 |
| 17. | acid. | 432 | 1027·0 | 10·935 | 0·5797 | 0·6297 | 0·4326 | 1·0<br>1·0 |
| Daily average..... | | *440* | *1028·5* | *11·526* | *0·6754* | *0·6499* | *0·5114* | |
| Average for the series... | | *453* | *1028·6* | *12·028* | *0·7292* | *0·6534* | *0·5070* | |

crease in the latter is always accompanied by an increase in the two former. In the grand average of the results, however, the slight increase in nitrogen is not accompanied by a corresponding increase in sulphur. In fact, the two series of results, indicate plainly that the antimony was without any material action. The total amount of antimony given, 16 grains of the oxide during 13 days, was certainly sufficient in quantity to have exerted its peculiar influence if possessed of any. The antimony was certainly absorbed, and that too in considerable amount. Thus on the 11th of June the 24 hours' urine contained 13·5 milligrams of metallic antimony; on the 17th, 22·4 milligrams; on the 18th, 17·6 milligrams and on the 20th of June, 15·1 milligrams of metallic antimony. These quantities of absorbed antimony would certainly indicate the presence of sufficient antimony for some decided influence on metabolic action, if any existed. The amount of nitrogen excreted daily, is seen to be considerably below the amount of nitrogen ingested. We did not make daily examinations of the fæcal matter, but such as were made showed plainly that the deficiency in nitrogen was contained mainly in the fæces; thus on the 5th of June the 100 grams of fæces excreted, contained 2·42 grams of nitrogen. At that date, the average amount of nitrogen excreted by the urine was 12·36 grams per day; this amount, added to the fæcal nitrogen makes a total of 14·78 grams excreted, against 15·25 grams ingested; a difference to be found mainly in the hair thrown off, and in part, in the ordinary errors of analysis.

We must conclude, therefore, that small repeated doses of antimonious oxide are without influence on the excretion of nitrogen, sulphur and phosphorus, and that consequently this compound, at least when taken in non-toxic doses, has no action on proteid metabolism.

## XX.—On Some Metallic Compounds of Albumin and Myosin.

By R. H. Chittenden and Henry H. Whitehouse, Ph.B.

Ever since Lieberkühn, in 1852, attempted to establish the molecular weight of albumin by preparing and analyzing the copper compound resulting from the action of a soluble copper salt on a solution of egg-albumin, various investigations have been published bearing on the nature and composition of the compounds of albumin with the heavy metals. Before this time even, F. Rose, in 1833, had published an analysis of a copper compound of albumin in which he had found from 1·50 to 1·70 per cent. of cupric oxide, and Mitscherlich, in 1837, published an analysis of a similar albumin compound, in which he found from 2·8 to 3·3 per cent. of cupric oxide, while Lieberkühn's compound contained 4·6 per cent. CuO. Compounds of albumin with other metals have also from time to time been prepared, such as zinc, lead, silver and mercury, and in one or two cases provisional formulæ have been given. The results, however, are to be considered as quite uncertain. With platinum chloride a compound appears to have been formed* of somewhat more certain composition. Aside from the more recent experiments of Ritthausen† on the vegetable albumins (gluten-casein, legumin, etc.), egg-albumin has been the chief albuminous body experimented with, and copper the main metal.

Recent work by one of us (C) on the albumose and globulose bodies, together with work on the products formed from casein and myosin, has led to a partial study of the metallic compounds of these bodies. As a preliminary, however, we found it necessary to study a few of the compounds of egg-albumin, and as the results thus obtained were not in accord with the more recent results of Harnack‡ we have continued our work with egg-albumin and with myosin, the results of which we now present.

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* See Commaile, Moniteur Scientifique, 1866, and Fuchs, in Annalen der Chemie, vol. cli, p. 372.

† Die Eiweisskörper der Getreidearten, etc. Journal für prakt. Chem., vol. xii, p. 361.

‡ Untersuchungen über die Kupferverbindungen des Albumins. Zeitschrift für physiologische Chemie, vol. v, p. 198.

## I. Egg-albumin.

### (a) *Copper Compounds.*

In looking over the literature of the subject, it becomes evident at once that the older investigators, owing either to the nature of the compound, to adherent impurities or to faulty methods, were not able to obtain concordant results, since the copper compound of egg-albumin, as prepared and analyzed by six distinct investigators, was found to contain from 1·50 to 5·19 per cent. of CuO. In all of these cases the preparation of the copper compound was essentially the same; a solution of egg-albumin was precipitated with a solution of a copper salt, the precipitate collected, washed thoroughly with water, dried, and the copper determined by simple ignition. Naturally this method, as suggested by Harnack, might be expected to give too high results, since the copper precipitate would unquestionably retain considerable of the inorganic matter of the albumin. Treated in this manner, however, F. Rose,* as already stated, found the copper compound to contain from 1·50 to 1·69 per cent. of CuO. Mitscherlich,† who held that the copper precipitate was a compound of egg-albumin with the copper salt, found in his preparations 2·8–3·3 per cent. CuO, while Bielitzki,‡ who demonstrated that the precipitate was an actual compound of albumin with cupric oxide, found in his preparations 4·75–5·20 per cent. of CuO. Lassaigne, as quoted by Harnack, found 4·95 per cent. of CuO, Mulder§ 4·44 per cent., while Lieberkühn's‖ preparation contained 4·6 per cent. of CuO. Further, Ritthausen's copper compounds of the vegetable albumins were found to contain from 11·5 to 17·0 per cent. of CuO. These results collectively, would therefore seem to indicate that when egg-albumin is precipitated by a soluble copper salt, the resulting compound does not contain a definite proportion of albumin and cupric oxide, or else that there are a large number of albumin-copper compounds. More recently, however, E. Harnack,¶ from analysis of fifteen separate preparations, comes to the conclusion that there are two distinct compounds of albumin with copper; one containing 1·35 per cent. of Cu, the other 2·64 per cent. of Cu, indicating as Harnack suggests, a copper albuminate in the first case of the formula $C_{204}H_{320}N_{52}O_{66}S_2Cu$, in which Cu replaces two atoms of hydrogen in the albumin molecule, and in the second case, an albuminate of the formula $C_{204}H_{316}N_{52}O_{66}S_2Cu_2$, in

* Poggendorff's Annalen, vol. xxviii, 1833.
† Müller's Archiv. for 1837, p. 91.
‡ Dissertation, Dorpat, 1853.
§ Physiologische Chemie, 1844–51.
‖ Poggendorff's Annalen, vol. lxxxvi, 1852.
¶ Loc. cit.

which two atoms of Cu replace four of hydrogen. The results obtained by this investigator would certainly seem to warrant this conclusion, for the analytical data of the different preparations show but slight variations; 1·34–1·37 per cent. in the one case, and 2·48–2·74 per cent. in the other. Further, Harnack worked with nearly ash-free preparations, the compounds after their first precipitation and washing being dissolved in sodium carbonate and reprecipitated by careful addition of acid. By repeating this process several times, the ash of the preparation was almost entirely removed, while the relative proportion of copper and albumin was not affected. As to the conditions which determine the formation of one or the other compound, there seems to be little definite other than that in general, the compound with smaller content of copper was obtained when the precipitation took place in the presence of a slight excess of albumin, and the compound with larger content of copper when in the presence of an excess of the copper salt. In no case were the copper salt and albumin solutions mixed in definite proportions, yet in every case one of the two compounds was formed; further, Harnack states that when an amount of copper salt exactly sufficient to form the albuminate is added to a given quantity of albumin, no precipitate results; in other words an excess of the copper salt is necessary to insure a separation of the compound.

Harnack's results, therefore, differ from those of the preceding investigators in that definite compounds appear to have been formed in every case, and further, in that the compounds contain a lower percentage of copper than found by any other investigators aside from F. Rose. This latter, it will be remembered, found 1·50–1·69 per cent. of CuO; 1·69 per cent. being equal to 1·34 per cent. of Cu, one of the percentages found by Harnack. Harnack further states that the average of the analyses made by other investigators, aside from Rose, show about 4·4 per cent. of CuO, and assuming that the various preparations contained an amount of ash equivalent to about 1 per cent. (which amount Harnack found in his preparations before purification) the percentage amount of cupric oxide would be reduced to about 3·4 =2·7 per cent. Cu, or the amount found by Harnack in his highest copper compound. But as Rose's preparation was made by the simple addition of an aqueous solution of egg-albumin to the copper salt and the copper determined as oxide by simple ignition, it would seem necessary to make the same deduction of 1 per cent. also in this case, which would make Rose's compound contain far less CuO than found

by Harnack. Further, Rose* found that the serum of
yielded a similar compound with cupric sulphate, which
only 1·14 per cent. of CuO or 0·88 per cent. of Cu. He
would seem to be little in these earlier investigations to su
the results obtained by Harnack. Mörner,† however, wor
alkali-albuminate, found that on precipitating a solution o
buminate with cupric sulphate, in the presence of an excess
he obtained a copper albuminate containing a percentage
oxide corresponding closely with that found by Lieberkühn
on the other hand, he precipitated a nearly neutral solution
albuminate with cupric sulphate, then the percentage of co
copper albuminate amounted to only one-third that found
kühn, or an amount about equivalent to that found by Harn
lowest copper compounds. Mörner further found that by
ing a calcium albuminate solution with cupric chloride, the
ate combined on an average with 2·33 per cent. of CuO, or
half the amount required by Lieberkühn's formula, and co
less than the amount contained in Harnack's largest co
pounds.

*Preparation of the albumin solution.*—In our experimen
thought best, as far as possible, to avoid exposing the albu
the action of alkalies, hence especial care was taken t
the egg-albumin as free from salts as possible, so that it
be necessary to purify the albuminate by reprecipitation. T
of a large number of eggs were finely divided by scissor
shaking with glass, then mixed with an equal volume of
thoroughly shaken with air, after which the solution wa
through cloth. Globulin was then precipitated by the
of dilute acetic acid (the acid added as long as a p
formed), the solution finally filtered through paper, after
filtrate was made exactly neutral with sodium carbonate
filtered. The fluid so obtained was then dialyzed in runn
for many days, a little thymol being added to prevent pu
The fluid finally obtained was perfectly neutral, clear and
but a small amount of inorganic salts.

In forming the albuminate we employed both cupric ac
cupric sulphate, using in each case the same volume of alb
tion, but varying the amount of copper salt. The copper
generally added as long as a precipitate formed. The

* Loc. cit., p. 139.

† Jahresbericht für Thierchemie,

ate was washed on a pump with water, until no reaction could be obtained with potassium ferrocyanide for copper or with acetic acid and potassium ferrocyanide for albumin. The preparations were dried at 100° C., then powdered and further dried at 110° C., until of constant weight. The percentage of copper was first determined by simple ignition and weighing as cupric oxide. The oxide was then dissolved in dilute nitric acid, the copper precipitated as sulphide with hydrogen sulphide and weighed as subsulphide by ignition in hydrogen gas with a little sulphur. Each series was made from a distinct preparation of albumin and nearly every compound made, was analyzed in duplicate.

Following are the analytical results:

### Series I.

*With* $CuSO_4$.

| No. | Am't sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu |
|---|---|---|---|---|---|
| 1*a* | 0·5621 gram. | 0·0081 gram. | 1·13 | 0·0070 gram. | 0·97 |
| *b* | 0·5176 | 0·0078 | 1·19 | 0·0065 | 0·98 |

*With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·5479 | 0·0088 | 1·27 | ---- | ---- |
| *b* | 0·5703 | 0·0091 | 1·26 | 0·0078 | 1·08 |

### Series II.

*With* $CuSO_4$.

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·5278 gram. | 0.0067 gram. | 1·00 | 0·0053 gram. | 0·79 |
| *b* | 0·7075 | 0·0083 | 0·93 | 0·0068 | 0·76 |

*With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·5697 | 0·0081 | 1·12 | 0·0071 | 0·98 |
| *b* | 0·5005 | 0·0073 | 1·15 | 0·0061 | 0·95 |

### Series III.

*With* $CuSO_4$.

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·6235 gram. | 0·0085 gram. | 1·07 | ---- | ---- |
| *b* | 0·6705 | 0·0091 | 1·07 | 0·0088 gram. | 1.04 |

*With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·8302 | 0·0130 | 1·24 | ---- | ---- |
| *b* | 0·9400 | 0·0151 | 1·27 | ---- | ---- |

## Series IV.

### *With* $CuSO_4$.

| No. | Amt. sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu. |
|---|---|---|---|---|---|
| 1*a* | 0·3621 gram. | 0·0047 gram. | 1·02 | 0·0038 gram. | 0·82 |
| *b* | 0·3478 | 0·0046 | 1·03 | 0·0039 | 0·89 |
| 2*a* | 0·4318 | 0·0060 | 1·08 | 0·0040 | 0·71 |
| *b* | 0·3288 | 0·0047 | 1·12 | 0·0033 | 0·78 |
| 3*a* | 0·4083 | 0·0060 | 1·15 | 0·0047 | 0·90 |
| *b* | 0·4332 | 0·0064 | 1·17 | 0·0053 | 0·96 |
| 4*a* | 0·3374 | 0·0047 | 1·09 | 0·0042 | 0·97 |
| *b* | 0·4289 | 0·0062 | 1·14 | 0·0050 | 0·90 |

### *With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 5*a* | 0·6358 | 0·0090 | 1·11 | 0·0073 | 0·91 |
| *b* | 0·5147 | 0·0071 | 1·08 | 0·0064 | 0·99 |
| 6*a* | 0·5321 | 0·0075 | 1·12 | 0·0065 | 0·95 |
| *b* | 0·5125 | 0·0073 | 1·13 | ---- | ---- |
| 7*a* | 0·7260 | 0·0125 | 1·36 | ---- | ---- |
| *b* | 0·6916 | 0·0120 | 1·37 | 0·0100 | 1·15 |

## Series V.

### *With* $CuSO_4$.

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·4838 gram. | 0·0083 gram. | 1·36 | 0·0074 gram. | 1·21 |
| *b* | 0·5083 | 0·0091 | 1·41 | ---- | ---- |

### *With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·5044 | 0·0085 | 1·32 | --- | ---- |
| *b* | 0·5042 | 0·0084 | 1·32 | ---- | ---- |

From the analyses of these 15 preparations it is to be seen that the percentage amount of metallic copper, determined as oxide by simple ignition, amounts on an average to 1·17 per cent. When, however, the copper is determined as subsulphide, by precipitation with hydrogen sulphide, and thus obtained free from ash, the percentage amount of copper falls on an average to 0·94 per cent. Cu. The preparations thus contain 0·23 per cent. of ash. We were not able to obtain any copper compounds with a much smaller content of ash than this, except by the use of methods which appear to affect the composition of the compound.

Harnack states* that he was able to obtain the copper albuminate quite free from ash by dissolving the freshly precipitated albuminate, after it had been thoroughly washed, in sodium carbonate, filtering and reprecipitating the compound by careful addition of acid. By repeating this process several times the adhering inorganic matter was entirely removed. It seemed questionable, however, whether this treatment might not induce some alteration in the compound.

The two following series of experiments were tried with the intention of throwing some light upon this point.

SERIES VI.

*With* $CuSO_4$.

| No. | Am't sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu. |
|---|---|---|---|---|---|
| 1*a* | 0·6320 gram. | 0·0091 gram. | 1·15 | 0·0080 gram. | 0·99 |
| *b* | 0·6980 | 0·0099 | 1·13 | 0·0086 | 0·98 |
| 2*a* | 0·5428 | 0·0077 | 1·12 | 0·0070 | 1·01 |
| *b* | 0·5121 | 0·0077 | 1·19 | 0·0061 | 0·95 |
| 3 | 0·3146 | 0·0080 | 2·03 | 0·0068 | 1·71 |
| | | *With* $Cu(C_2H_3O_2)_2$. | | | |
| 4*a* | 0·6105 | 0·0085 | 1·11 | 0·0075 | 0·96 |
| *b* | 0·5836 | 0·0078 | 1·06 | 0·0072 | 0·99 |
| 5*a* | 0·5996 | 0·0087 | 1·15 | 0·0081 | 1·06 |
| *b* | 0·5592 | 0·0082 | 1·16 | 0·0069 | 0·99 |
| 6 | 0·4139 | 0·0131 | 2·51 | 0·0115 | 2.19 |

In this series of experiments all of the preparations, as before, were made from the purified albumin. Nos. 1, 2, 4 and 5 were, after precipitation, simply washed with water until the washings gave no reaction either for copper or albumin. No. 3, after being washed in a similar manner, was dissolved in very dilute sodium carbonate and reprecipitated by neutralization with dilute hydrochloric acid. No. 6 was dissolved up twice in this manner and both preparations were finally washed free from chlorine. A glance at the analyses shows plainly that this treatment has tended to increase the percentage amount of copper in the albuminate; due, doubtless, either to withdrawal of a portion of the albumin by the sodium carbonate, or else to a partial dissociation of the compound by the long continued washing with water.

The first reprecipitation has apparently increased the amount of copper in the compound fully 0·7 per cent., the second reprecipitation 0·5 per cent. more.

* Loc. cit., p. 202.

## Series VII.

### *With* $CuSO_4$.

| No. | Am't sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu. |
|---|---|---|---|---|---|
| 1*a* | 0·6326 gram. | 0·0091 gram. | 1·13 | ---- | ---- |
| *b* | 0·6156 | 0·0086 | 1·10 | ---- | ---- |
| 2*a* | 0·4054 | 0·0081 | 1·60 | 0·0063 gram. | 1·23 |
| *b* | 0·4995 | 0·0099 | 1·58 | 0·0074 | 1·19 |
| 3*a* | 0·3870 | 0·0068 | 1·39 | 0·0049 | 1·02 |
| *b* | 0·3702 | 0·0066 | 1·40 | ---- | ---- |

### *With* $Cu(C_2H_3O_2)_2$.

| | | | | | |
|---|---|---|---|---|---|
| 4*a* | 0·6175 | 0·0085 | 1·08 | 0·0074 | 0·95 |
| *b* | 0·6069 | 0·0082 | 1·07 | 0·0077 | 1·00 |
| 5*a* | 0·3282 | 0·0072 | 1·73 | 0·0055 | 1·34 |
| *b* | 0·3755 | 0·0083 | 1·75 | ---- | ---- |
| 6*a* | 0·7503 | 0·0132 | 1·39 | 0·0104 | 1·10 |
| *b* | 0·7016 | 0·0121 | 1·38 | ---- | ---- |

This series was prepared in the same manner as the preceding. Nos. 1, 3, 4 and 6 were simply washed with water, while No. 2 was reprecipitated once and No. 5 twice, and both ultimately washed free from all soluble matters. The results show here the same increased percentage of copper, although not so marked as in the preceding series, when the albuminate is dissolved in sodium carbonate and reprecipitated. Further, the percentage of ash is not, as a rule, materially changed by this process; thus in No. 2, where the albuminate was reprecipitated once, the difference in the percentage of copper as determined by simple ignition and by precipitation as sulphide, amounts to 0·37 per cent., while in No. 3*a*, where the compound was not reprecipited at all, the difference is exactly the same.

In precipitating the albuminate, there is formed in the fluid a small amount of either sulphuric or acetic acid. Harnack, to avoid this, states that it is better, after adding the necessary amount of cupric sulphate to the albumin solution, to exactly neutralize the mixture with sodium carbonate. If, however, the greatest care is not exercised and excess of cupric sulphate avoided, even partial neutralization of the fluid will result in the precipitation of a portion of the copper and thus show an apparent increase in the copper of the albuminate. So far, however, as our results show, the small amount of sulphuric acid liberated in the formation of the albuminate does not affect the character of the compound. In the following series, after each precipitation,

the mixture was made as near neutral as possible with dilute sodium carbonate and the compounds then filtered and washed thoroughly with water. The copper in this series was determined simply by ignition.

SERIES VIII.

*With* $CuSO_4$.

| No. | Am't sub. taken. | Wt. CuO. | Per cent. Cu. |
|---|---|---|---|
| 1*a* | 0·4273 gram. | 0.0068 gram. | 1·26 |
| *b* | 0·3867 | 0·0063 | 1·29 |
| 2*a* | 0·3167 | 0·0049 | 1·23 |
| *b* | 0·3535 | 0·0052 | 1·18 |
| 3*a* | 0·2418 | 0·0030 | 1·00 |
| *b* | 0·3079 | 0·0039 | 1·00 |
| 4*a* | 0·6210 | 0·0138 | 1·78 |
| *b* | 0·7091 | 0·0158 | 1·78 |

The results plainly show no appreciable difference in the composition of the albuminate under this change in the conditions, unless in No. 4 where a larger amount of copper is found than usual. It is our opinion, however, that the small amount of acid liberated by the reaction is not sufficient to cause any especial change in the character of the albuminate; neither, probably, does very dilute sodium carbonate in itself change the substance to such an extent that on neutralization it is not precipitated in nearly its original form, or at least that the action in this case is not any greater than that produced by water alone. In fact we are much inclined to the view that the long continued action of water will gradually but surely affect the composition of the albuminate, and that doubtless the change in the composition of the compound noticed in our experiments on solution of the substance in sodium carbonate and reprecipitation is due to the combined action of the alkaline fluid and of water. Harnack states that week-long treatment of the freshly precipitated albuminate with water will gradually cause dissociation of the compound, but that it can be easily and thoroughly washed without any decomposition whatever.

Our experience, however, leads us to question the correctness of this view. Ordinarily, it has taken us an entire day to completely wash the freshly precipitated albuminate, so that the wash-water should give no reaction whatever for copper or albumin. In precipitating the albumin solution with cupric sulphate, the albumin never appears to be completely precipitated and at the same time, as Harnack has observed, it is necessary to add more than the proportional

amount of copper salt to obtain any separation of the albuminate. As a result, the filtrate contains considerable albumin and copper, but even after several hours washing on the pump (the filtration is slow at the best) the precipitate still gives up traces of albumin, as shown by acetic acid and potassium ferrocyanide, long after all traces of copper have disappeared. It is not impossible to wash the compound and reach a point where the wash-water contains neither copper nor albumin, but when the washing goes on slowly and the water remains more or less in contact with the albuminate for 24 hours, then frequently the washings will show traces of albumin continuously, without our being able to reach a point where the test fails to give any reaction whatever, or to show any special change in the intensity of the reaction.

The following series of experiments would appear to substantiate this view. The first six were washed for about twelve hours, when no copper reaction could be obtained in the washings and only the slightest reaction for albumin. The last six were washed for sixteen hours, and finally stood over night on wet filters with more or less water on them. At the end of this time, the washings continued to show a reaction for albumin with acetic acid and potassium ferrocyanide, and indeed the reaction appeared to increase rather than diminish in intensity on further washing. The washings contained no copper.

Following are the results of the analyses:

SERIES IX.—*With* $CuSO_4$.

| No. | Amt. sub. taken. | Wt. CuO. | Per cent. Cu. |
|---|---|---|---|
| 1 | 0·4207 gram. | 0·0052 gram. | 0·99 |
| 2 | 0·4676 | 0·0058 | 0·98 |
| 3 | 0·4881 | 0·0062 | 1·00 |
| 4 | 0·3162 | 0·0037 | 0·91 |
| 5 | 0·2882 | 0·0032 | 0·89 |
| 6 | 0·1857 | 0·0024 | 1·02 |
| 7 | 0·2892 | 0·0050 | 1·34 |
| 8 | 0·4137 | 0·0060 | 1.13 |
| 9 | 0·4077 | 0·0060 | 1.18 |

*With* $Cu(C_2H_3O_2)_2$.

| | | | |
|---|---|---|---|
| 10 | 0·6067 | 0·0126 | 1·64 |
| 11 | 0·6889 | 0·0111 | 1·27 |
| 12 | 0·7126 | 0·0109 | 1·22 |

While the difference is not very great, it is a constant difference, and it is to be remembered that the last six compounds differ in no respects whatever from the first six, except in being subjected to the longer action of water.

In comparing now these different results, it is seen that we have not been able to obtain a copper albuminate with a higher content of Cu than 2·19 per cent., and this only as a result of two reprecipitations; a condition, which, from our experience, tends to alter materially the composition of the original precipitate. The average of the results obtained by simple precipitation, show a content of 0·94 per cent. of Cu. A study of the individual results, however, shows too great a variation to believe wholly in the existence of a single, stable copper albuminate. Either there are one or two definite compounds, which, being more or less unstable, are prone to change under varying conditions and thus give rise to the variations in the content of copper noticed, or else there are a number of definite compounds liable to be formed as the conditions are varied, all of which, however, must be more or less unstable. Glancing over the individual results, it is plain that an amount of Cu approximating to 0·96 per cent. is found altogether too frequently to be the result of chance. Doubtless this figure represents most closely the content of copper in the ordinary copper albuminate obtained by simple precipitation, while the majority of the variations from this figure are due mainly to dissociation.

Taking Lieberkühn's formula of albumin, the following copper albuminates would be possible:

$(C_{72}H_{112}N_{18}SO_{22})_3 + Cu - H_2 = 1{\cdot}29$ per cent. Cu.
$(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2 = 0{\cdot}96$ " " "
$(C_{72}H_{112}N_{18}SO_{22})_6 + Cu - H_2 = 0{\cdot}77$ " " "

For the first, in which Lieberkühn's formula for albumin is trebled, the percentage of copper corresponds nearly to the lowest results obtained by Harnack, while in the second formula the percentage of copper accords closely with the average of our results. Whether the weight of the albumin molecule is represented more nearly by the second formula than by the first we have not sufficient data to determine, but certainly our results with the copper albuminate show a lower percentage of copper than would correspond with the first formula. Further, it would appear that the copper albuminates are readily prone to change under slight provocation and that this point, in part, undoubtedly explains the reason for the great variation in the results obtained by so many workers.

### (b) *Lead Compounds.*

Lieberkühn* states that the lead salt of albumin cannot be obtained pure; that the insoluable precipitate formed by the addition of either lead nitrate or basic lead acetate to a solution of albumin, is simply a mixture. With basic lead acetate, Lieberkühn obtained a precipitate containing 17·86 per cent. of lead oxide, while the precipitate formed with lead nitrate contained 12·78 per cent. of lead oxide.

With protein, Mulder† obtained precipitates on the addition of neutral lead acetate and lead nitrate, which contained respectively 12·45 and 12.68 per cent. of lead oxide, while basic lead acetate gave a precipitate containing 30·63 per cent. of lead oxide. Berzelius‡ states that neutral lead acetate precipitates both albumin and blood serum, but that the greater portion of the albumin remains dissolved in the fluid united with acetic acid. Basic lead acetate on the other hand precipitates the albumin completely.

These last statements accord with our own results; with a neutral lead salt only a small precipitate was obtained, the compound being soluble apparently in both excess of the lead salt and of albumin, while with basic lead acetate the albumin seemed completely precipitated.

Further, Berzelius‖ states, on the authority of Mulder, that if a solution of potassium albuminate be made as neutral as possible with acetic acid and then precipitated with lead nitrate, the lead albuminate so obtained contains on thorough drying 5·84 per cent. of lead oxide.

Following are some of the results of our analyses. The compounds were made from thoroughly dialyzed albumin and were washed free from both lead and any excess of albumin. The preparations were dried at 110° C. until of constant weight and the lead was determined first by simple ignition, with addition of a little ammonium nitrate. The lead oxide, after being weighed, was then dissolved in dilute nitric acid, the solution evaporated to a small volume, the lead precipitated with a little sulphuric acid, two volumes of alcohol added, and the lead sulphate finally filtered and washed with 95 per cent. alcohol. The sulphate was then ignited with proper precautions and from the weight obtained, the percentage of lead again calculated.

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* Poggendorff's Annalen, Band lxxxvi, p. 124.

† Lehrbuch der Chemie, Berzelius, ix, p. 29. ‡ Lehrbuch der Chemie, ix, p. 43.

‖ Lehrbuch, ix, p. 49.

### Series I.

*With neutral lead acetate.*

| No. | Amt. Sub. taken. | Wt. PbO. | Per cent. Pb. | Wt. $PbSO_4$. | Per cent. Pb. |
|---|---|---|---|---|---|
| 1*a* | 0·4972 gram. | 0·0188 gram. | 3·49 | 0·0209 gram. | 2·85 |
| *b* | 0·3992 | 0·0150 | 3·48 | 0·0166 | 2·83 |

### Series II.

*With neutral lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·5341 | 0·0183 | 3·16 | 0·0216 | 2·75 |
| *b* | 0·5381 | 0·0198 | 3·40 | 0·0222 | 2·80 |

*With basic lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·5832 | 0·0460 | 7·30 | 0·0580 | 6·77 |
| *b* | 0·5008 | 0·0391 | 7·22 | 0·0487 | 6·62 |

### Series III.

*With neutral lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·8057 | 0·0262 | 3·01 | 0·0274 | 2·32 |
| *b* | 0·6522 | 0·0211 | 2·98 | 0·0226 | 2·36 |

*With basic lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·7290 | 0·0593 | 7·55 | 0·0615 | 5·74 |
| *b* | 0·7630 | 0·0631 | 7·66 | 0·0609 | 5·45 |

### Series IV.

*With neutral lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·4121 | 0·0152 | 3·42 | 0·0136 | 2·25 |
| *b* | 0·4823 | 0·0176 | 3·37 | 0·0178 | 2·50 |

*With basic lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 2*a* | 0·7234 | 0·0651 | 8·34 | ------ | ---- |
| *b* | 0·5365 | 0·0482 | 8·33 | ------ | ---- |

### Series V.

*With a large excess of basic lead acetate.*

| | | | | | |
|---|---|---|---|---|---|
| 1*a* | 0·6822 | 0·2119 | 28·81 | ------ | ---- |
| *b* | 0·5429 | 0·1714 | 29·28 | ------ | ---- |
| 2*a* | 0·5836 | 0·1923 | 30·56 | ------ | ---- |
| *b* | 0·5918 | 0·1960 | 30·76 | ------ | ---- |
| 3*a* | 0·5478 | 0·1896 | 32·11 | ------ | ---- |
| *b* | 0·5427 | 0·1873 | 32·02 | ------ | ---- |

These results plainly indicate that more than one compound of lead is formed, especially so with basic lead acetate, the composition being dependent in this case on the amount of lead salt added. With neutral lead acetate, the variations in composition are not so marked and as it is hardly possible to prepare a lead albuminate free from salts, or to eliminate them wholly in the calculations, it is questionable how far the results should be trusted, except in a general way.

The formula $(C_{72}H_{112}N_{18}SO_{22})_4+Pb-H_2$ would require 3·10 per cent. Pb, while $(C_{72}H_{112}N_{18}SO_{22})_5+Pb-H_2$ would require 2·50 per cent. Pb. In the case of the albuminate formed with basic lead acetate, it is to be noticed that the compound made by the addition of a large excess of the lead salt, contains about five times as much lead as the ordinary basic lead compounds.

### (c) *Iron Compounds.*

F. Rose* has made iron albuminate, both from egg-albumin and from the serum of ox-blood, by the simple addition of ferric chloride to the albumin solution. Two preparations made from egg-albumin yielded respectively 2·79 and 2·88 per cent. of ferric oxide. Rose found the albuminate, when freshly precipitated, easily soluble both in excess of ferric chloride and in excess of the albumin solution.

Our preparations were made wholly from dialyzed albumin, and when so prepared and thoroughly washed the compound was found almost wholly free from adhering salts, so much so that after a few trials we deemed it unnecessary to make the determinations of iron other than by simple ignition and weighing as ferric oxide.

Following are some of our results:

SERIES I.

*With* $Fe_2Cl_6$.

| No. | Amt. sub. taken. | Wt. $Fe_2O_3$. | Per cent. $Fe_2O_3$. | Per cent. Fe. |
|---|---|---|---|---|
| 1*a* | 0·7033 gram. | 0 0094 gram. | 1·33 | 0·92 |
| *b* | 0·6430 | 0·0086 | 1·33 | 0·93 |

SERIES II.

| | | | | |
|---|---|---|---|---|
| 1*a* | 0·4683 | 0·0052 | 1·11 | 0·76 |
| *b* | 0·3854 | 0·0042 | 1·08 | 0·75 |

* Poggendorff's Annalen, xxviii. p. 140, 1833.

SERIES III.

| No. | Amt. sub. taken. | Wt. $Fe_2O_3$. | Per cent. $Fe_2O_3$. | Per cent. Fe. |
|---|---|---|---|---|
| 1*a* | 0·4710 | 0·0071 | 1·50 | 1·04 |
| *b* | 0·5329 | 0·0083 | 1·55 | 1·08 |
| 2*a* | 0·7387 | 0·0100 | 1·36 | 0·94 |
| *b* | 0·6355 | 0·0087 | 1·36 | 0·94 |

SERIES IV.

| | | | | |
|---|---|---|---|---|
| 1*a* | 0·4862 | 0·0063 | 1·30 | 0·90 |
| *b* | 0·5558 | 0·0073 | 1·31 | 0·91 |
| 2*a* | 0·5115 | 0·0070 | 1·36 | 0·95 |
| *b* | 0·4954 | 0·0066 | 1·33 | 0·92 |
| 3*a* | 0·4505 | 0·0063 | 1·39 | 0·97 |
| *b* | 0·4610 | 0·0066 | 1·43 | 0·99 |
| 4*a* | 0·4817 | 0·0071 | 1·47 | 1·01 |
| *b* | 0·4571 | 0·0065 | 1·44 | 0·98 |
| 5*a* | 0·4819 | 0·0065 | 1·34 | 0·93 |
| *b* | 0·3846 | 0·0051 | 1·33 | 0·93 |
| 6*a* | 0·4086 | 0·0059 | 1·45 | 1·00 |
| *b* | 0·3392 | 0·0048 | 1·41 | 0·97 |
| 7*a* | 0·3814 | 0·0050 | 1·32 | 0·91 |
| *b* | 0·4107 | 0·0058 | 1·29 | 0·90 |
| 8*a* | 0·4452 | 0·0058 | 1·30 | 0·91 |
| *b* | 0·4833 | 0·0065 | 1·34 | 0·92 |

These results show a fairly close agreement with one single exception, in which case the percentage of iron is nearly 0·25 below the average. The average percentage, moreover, of ferric oxide is just about one-half that found by Rose. Further, the average percentage of iron (Fe) corresponds very closely with the average percentage of Cu in the copper albuminate. Eliminating one compound with only 0·75 per cent. of iron, the average content is seen to be 0·95 per cent.

$$(C_{72}H_{112}N_{18}SO_{22})_4 + Fe - H_2 = 0{\cdot}86 \text{ per cent. Fe.}$$
$$(C_{72}H_{112}N_{18}SO_{22})_3 + Fe - H_2 = 1{\cdot}14 \text{ per cent. Fe.}$$

As the iron was determined by simple ignition it would be expected that the amount found would exceed the theoretical amount somewhat; hence the first formula, assuming Lieberkühn's formula to be correct, would be more closely in accord with our results. The results obtained indicate further, that the iron albuminate is a much more stable compound than the copper albuminate, less liable to change and less readily affected by water.

### (d) *Zinc Compounds.*

With zinc we made but a few experiments and those mainly to see whether the low percentage of iron found in the iron albuminate would be substantiated by a corresponding percentage of zinc in the zinc albuminate. Lieberkühn has prepared and analyzed a zinc albuminate, made by the action of zinc sulphate on a neutral solution of alkali albuminate, and he found the compound to contain 4·66 per cent. of zinc oxide.

Our preparations were made by the action of a similar zinc salt on a solution of purified and dialyzed albumin. Following are the results obtained with two preparations made from two distinct lots of albumin:

| No. | Amt. sub. taken. | Wt. ZnO. | Per cent. ZnO. | Per cent. Zn. |
|---|---|---|---|---|
| 1*a* | 0·2424 gram. | 0·0031 gram. | 1·27 | 0·98 |
| *b* | 0·2838 | 0·0034 | 1·21 | 0·97 |
| 2*a* | 0·2166 | 0·0023 | 1·06 | 0·83 |
| *b* | 0·2354 | 0·0025 | 1·08 | 0·86 |

The average of these two results shows a composition proportional to that found in the case of the iron albuminate and suggests plainly that if we have to deal in these cases with a single albuminate of constant composition, the percentage amount of metal is much smaller than formerly was supposed.

Further, the percentage of zinc found accords closely with the theoretical amount for a zinc albuminate formed on the type of the copper compound.

$$(C_{72}H_{112}N_{18}SO_{22})_4 + Zn - H_2 = 0{\cdot}99 \text{ per cent. Zn.}$$

### (e) *Uranium Compounds.*

N. Kowalewsky* has recently called attention to the use of uranic or uranyl acetate as a reagent for albuminous matter, and has shown that it is not only a good precipitant of albumin at ordinary temperatures, but also that it is an extremely delicate one. Further, Kowalewsky states that the uranyl-albumin compound on ignition leaves a dark, olive green ash, composed of the green uranoso-uranic oxide, $U_3O_8$. A determination of the amount of this ash in several preparations showed 12·09 to 13·4 per cent., presumably of $U_3O_8$.

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* Essigsaures Uranoxyd, ein Reagens auf Albuminstoffe. Zeitschrift für Analytische Chemie, 1885, p. 551.

Our preparations were made by adding uranyl nitrate to the prepared albumin solution and washing the precipitated albuminate until all excess of uranium was removed. The uranium in the dried preparation was determined as uranoso-uranic oxide ($U_3O_8$) by simple ignition. The results show a fairly close agreement, but they are undoubtedly somewhat too high, owing to a small amount of adherent ash.

*With* $UO_2(NO_3)_2$.

| No. | Amt. sub. taken. | Wt. $U_3O_8$. | Per cent. $U_3O_8$. | Per cent. U. |
|---|---|---|---|---|
| 1*a* | 0·5980 gram. | 0·0324 gram. | 5·41 | 4·59 |
| *b* | 0·5193 | 0·0281 | 5·41 | 4·59 |
| 2*a* | 0·8219 | 0·0428 | 5·20 | 4·41 |
| *b* | 0·8081 | 0·0418 | 5·17 | 4·38 |
| 3*a* | 0·4392 | 0·0251 | 5·71 | 4·84 |
| *b* | 0·5330 | 0·0303 | 5·68 | 4·81 |
| 4*a* | 0·8183 | 0·0427 | 5·22 | 4·43 |
| *b* | 0·7576 | 0·0394 | 5·20 | 4·41 |
| 5 | 0·6985 | 0·0367 | 5·25 | 4·46 |
| 6*a* | 0·4269 | 0·0247 | 5·78 | 4·90 |
| *b* | 0·5496 | 0·0318 | 5·70 | 4·83 |

These results plainly do not accord at all with Kowalewsky's. On the other hand they do agree fairly well with each other, and would seem to indicate a reasonably constant composition of the uranyl-albumin precipitate.

The average of the results obtained, accords most closely with the formula

$$(C_{72}H_{112}N_{18}SO_{22})_3 + U - H_6$$

which requires 4·73 per cent. U.

### (f) *Mercury compounds.*

By the addition of an excess of mercuric chloride solution to an aqueous solution of egg-albumin, an albuminate of mercury is formed, insoluble in excess of the mercury salt. The compound can be easily filtered and admits of thorough washing with water. Rose first proved that the precipitate formed as above, is a compound of mercury with albumin, instead of a compound of the mercury salt with albumin as supposed by Bostock and Orfila.

We have made a few preparations of the albuminate by adding a moderately strong aqueous solution of mercuric chloride to portions of the dialyzed albumin solution and washing the precipitates thoroughly with water. The mercury in the albuminate was deter-

mined by ignition in a combustion tube with quick lime, with a posterior layer of calcium carbonate and sodium bicarbonate.*

The mercury distilled, was collected in water and after thorough washing with alcohol to remove hydrocarbons, etc., was dried and weighed.

Following are the results of our analyses of the several preparations made.

| No. | Amt. Sub. taken. | Wt. Hg. | Per cent. Hg. |
|---|---|---|---|
| 1*a* | 0·8050 gram. | 0·0226 gram. | 2·80 |
| *b* | 0·8732 | 0·0274 | 3·13 |
| 2*a* | 0·7637 | 0·0215 | 2·82 |
| *b* | 0·7357 | 0·0201 | 2·73 |
| 3*a* | 0·5088 | 0·0150 | 2·96 |
| *b* | 0·6261 | 0·0167 | 2·66 |
| 4*a* | 0·9152 | 0·0300 | 3·28 |
| *b* | 0·8503 | 0·0270 | 3·17 |
| 5*a* | 0·8492 | 0·0218 | 2·56 |
| *b* | 0·8610 | 0·0237 | 2·75 |
| 6*a* | 0·9674 | 0·0284 | 2·93 |

The average content of mercury is 2·89 per cent. The theoretical amount for $(C_{12}H_{112}N_{18}SO_{22})_4 + Hg - H_2$ is 3·00 per cent.

### (g) *Silver compounds.*

Silver nitrate is a well known precipitant of albumin, and Lieberkühn,† many years ago, assigned to silver albuminate a definite formula, calling for 6·67 per cent. of silver oxide. The preparation made by him from egg-albumin was found to contain 6·55 per cent. of silver oxide = to 6·27 per cent. of Ag. Mulder,‡ likewise, working with alkali-albuminate, found that by neutralizing the solution as nearly as possible with acetic acid, and then precipitating with silver nitrate, the silver albuminate so prepared contained 6·14 per cent. of silver oxide.

Fuchs§, using ordinary egg-albumin instead of alkali-albuminate, found only half as much silver (3·28 per cent. Ag), while O. Loew,‖ working with purified egg-albumin, found still smaller percentages of silver in the albuminate made by him. Using an albumin

* See Fresenius, Quantitative Chemical Analysis.

† Poggendorff's Annalen, 1852, vol. clxii, p. 123.

‡ See Berzelius' Lehrbuch der Chemie, vol. ix, p. 49.

§ Annalen d. chem. u. Pharm., Band cli, p. 372.

‖ Pflüger's Archiv für Physiologie, Band xxxi, p. 393; Ueber Eiweiss und Pepton

solution purified simply by three days' dialysis, Loew found that a 1 per cent. solution of silver nitrate gave no precipitate whatever in a 5 per cent. solution of albumin. On adding a little dilute sulphuric acid to the albumin solution, however, and then pouring the mixture into the silver solution a precipitate was obtained, which on thorough washing and drying was found to contain 2·17 per cent. of Ag; while a second preparation made by using a little less sulphuric acid contained 2·40 per cent. of Ag. By precipitating the albumin solution directly with a 5 per cent. solution of silver nitrate, without the addition of any acid, the albuminate was found to contain in one case 4·39 per cent. Ag, in a second case 3·91 per cent. Ag. Dissolving the freshly precipitated albuminate formed in this manner, in dilute ammonia and then reprecipitating it by the addition of dilute sulphuric acid to slight acid reaction, the albuminate was found to contain 4·64 per cent. of Ag.

Loew sees in these results a confirmation of Harnack's views as to the copper albuminates, and an assurance that the molecular weight of albumin corresponds to Lieberkühn's formula three times enlarged.

Using an albumin solution purified as in our previous experiments and adding to it a 10 per cent. solution of silver nitrate as long as a precipitate was formed, four distinct series of albuminates were made* representing four distinct preparations of egg-albumin. These were all washed free from silver and also from any adhering albumin, dried at 110° C. until of constant weight and the silver determined by simple ignition.

SERIES I.

| No. | Amt. Sub. taken. | Wt. Ag. | Per cent. Ag. |
|---|---|---|---|
| 1*a* | 0·5900 gram. | 0·0242 gram. | 4·10 |
| *b* | 0·5625 | 0·0230 | 4·08 |
| 2*a* | 0·5760 | 0·0230 | 4·11 |
| *b* | 0·7548 | 0·0305 | 4·04 |
| 3*a* | 0·9005 | 0·0362 | 4·02 |
| *b* | 0·7973 | 0·0325 | 4·07 |

SERIES II.

| | | | |
|---|---|---|---|
| 1*a* | 0·5859 | 0·0245 | 4·18 |
| *b* | 0·6967 | 0·0290 | 4·16 |
| 2*a* | 0·9473 | 0·0385 | 4·06 |
| *b* | 0·6621 | 0·0270 | 4·07 |
| 3*a* | 0·6455 | 0·0266 | 4·12 |
| *b* | 0·7000 | 0·0285 | 4·07 |

* The silver compounds were all made and analyzed by Mr. T. S. Bronson of this laboratory.

SERIES III.

| | | | |
|---|---|---|---|
| 1*a* | 0·5860 | 0·0239 | 4·07 |
| *b* | 0·6949 | 0·0284 | 4·08 |
| 2*a* | 0·7090 | 0·0290 | 4·09 |
| *b* | 0·9053 | 0·0374 | 4·13 |
| 3*a* | 0·5980 | 0·0247 | 4·13 |
| *b* | 0·8000 | 0·0328 | 4·10 |

SERIES IV.

| | | | |
|---|---|---|---|
| 1*a* | 0·6217 | 0·0300 | 4·88 |
| *b* | 0·6810 | 0·0331 | 4·86 |
| 2*a* | 0·5509 | 0·0270 | 4·90 |
| *b* | 0·5626 | 0·0278 | 4·86 |
| 3*a* | 0·6955 | 0·0396 | 5·69 |
| *b* | 0·7515 | 0·0430 | 5·72 |

The figures show a far smaller content of silver in all of the preparations than found by Lieberkühn or Mulder. In three of the series, there is seen a constancy of composition which is quite noticeable and, further, a close agreement with the second result obtained by Loew on adding a 5 per cent. solution of silver nitrate to the albumin. In the last series, however, the percentage of silver is somewhat higher, possibly owing to incomplete dialysis of the chlorides and phosphates from the albumin solution. These figures, however, are not much higher than the highest figures obtained by Loew.

A silver salt of albumin, of the composition $(C_{72}H_{112}N_{16}SO_{22})$, $+Ag_2-H_2$ would contain 4·28 per cent. of Ag, and while our results certainly approximate to this figure, there is variation enough to indicate an equal possibility of a mixture of two or more compounds. With a molecule of the size of the albumin molecule, it is possible by doubling or otherwise, to obtain a formula corresponding to almost any percentage of metal found. And inasmuch as every variation* in the method of preparing the albuminate tends to alter its composition, it seems worse than useless at present, to lay much stress on the exact constitution of the silver albuminate. A large number of albuminates are of course possible, but until we know more definitely how to separate one from another, we have no guarantee of the simple nature of any one.

* Loew states that he has prepared a silver albuminate containing 10·7 per cent Ag, corresponding nearly to 6 atoms of silver, and that it is possible to prepare albuminates still richer in silver.

Examining now, all of the results obtained, we find the following average composition of the albuminates studied :

| | | | | |
|---|---|---|---|---|
| Copper compound, | 0·94 | per | cent. | Cu |
| Iron compound, | 0·95 | " | " | Fe* |
| Zinc compound, | 0·91 | " | " | Zn |
| Lead (neutral salt) compound, | 2·56 | " | " | Pb |
| Uranyl compound, | 4·60 | " | " | U |
| Silver compound, | 4·09 | " | " | Ag† |
| Mercury compound, | 2·89 | " | " | Hg |

Accepting Lieberkühn's formula of albumin as correct, then the following formulæ accord most closely with the above percentages.

| | | | | |
|---|---|---|---|---|
| $(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2$ | requires | 0·96 | per cent. | Cu |
| $(C_{72}H_{112}N_{18}SO_{22})_4 + Fe - H_2$ | " | 0·86 | " | Fe |
| $(C_{72}H_{112}N_{18}SO_{22})_4 + Zn - H_2$ | " | 0·99 | " | Zn |
| $(C_{72}H_{112}N_{18}SO_{22})_3 + Pb - H_2$ | " | 2·50 | " | Pb |
| $(C_{72}H_{112}N_{18}SO_{22})_3 + U - H_6$ | " | 4·73 | " | U |
| $(C_{72}H_{112}N_{18}SO_{22})_3 + Ag_4 - H_2$ | " | 4·28 | " | Ag |
| $(C_{72}H_{112}N_{18}SO_{22})_4 + Hg - H_2$ | " | 3·00 | " | Hg |

We do not, however, lay much stress upon the accuracy of these formulæ. The results obtained in our study of these metallic compounds do by chance accord with them, and inasmuch as Loew and Harnack are disposed to treble Lieberkühn's formula for albumin, on the basis of the composition of the copper and silver albuminates, made by them respectively, we present our results as evidence that there are equally good grounds for quadrupling the above formula.

We believe, however, that with the majority of these albuminates it is possible to form a large variety of compounds with the same metal, by simply modifying the conditions of precipitation. This is evidenced by Loew's results with silver albuminate and our own with lead and copper, and since a great variety of compounds are possible, it is equally possible that in many cases we may have to do with mixtures of such compounds, which would account for the great variability in composition noticed in some of the albuminates and for the lack of agreement in the results obtained by different workers. Coupled with this, in some cases, is the undoubted tendency of the compounds to dissociation.

## II. *Myosin.*

The myosin employed was prepared from ox flesh, by extraction with a 15 per cent. solution of ammonium chloride, after the tissue

* Excepting one very low result. † Excepting the last series of compounds.

had been thoroughly freed from salts and soluble albumin by long continued extraction with water. The myosin was separated from the ammonium chloride solution by dialysis, being obtained in this manner as a semi-gelatinous mass, readily soluble in salt solutions. In order to form compounds with the various metals, it was found best to use a solution of myosin in 5 per cent. ammonium chloride, the metallic compound when formed being washed with water until the washings gave no reaction for chlorine with silver nitrate. The compounds were then dried, first at 100° C., then at 110° C., until of constant weight. Control experiments with the metallic salt and ammonium chloride alone, invariably failed to give any precipitate whatever.

No systematic attempt has apparently been made to study any of the metallic compounds of myosin; in fact, few statements are to be found regarding the existence of such compounds. Danilewsky* some time ago, showed that myosin would combine with free mineral acids, uniting with them so that with tropæolin 00 no reaction for free acid could be obtained. With strong bases, however, according to Danilewsky, myosin does not probably combine, and the statement is further made that a small amount of calcium oxide ordinarily exists loosely combined with myosin, which calcium by coagulation of the myosin is liberated. Further, Danilewsky found that on adding platinum chloride in excess, to a dilute hydrochloric acid solution of myosin, a myosin-platinum chloride compound was precipitated, which after washing with water and alcohol and then drying at 100–105° C., contained 9·46 per cent. of platinum and 7·26 per cent. of chlorine. With copper, iron and similar salts we have not been able to obtain any precipitate in a hydrochloric acid solution of myosin. By adding, however, a solution of a metallic salt of such a nature that it does not react with ammonium chloride, to an ammonium chloride solution of myosin, a precipitate is produced, which as our experiments show, is ordinarily a compound of myosin with the metal or metallic oxide. This is readily seen by adding either zinc sulphate or ferric chloride to such a solution of myosin and then washing the precipitates with water, until the washings give no reaction for chlorine or for sulphuric acid. On now warming the iron precipitate with dilute nitric acid, a solution will be obtained, giving a distinct iron reaction but no reaction with silver nitrate for chlorine. Similarly on warming the zinc pre-

* Zeitschrift für physiologische Chemie, v, p. 160.

cipitate with hydrochloric acid, the solution gives no reaction for sulphuric acid with barium chloride.

With cupric sulphate and cupric acetate the same is ordinarily true. It is possible, however, to prepare a myosin-copper compound in which cupric sulphate appears to unite directly with the myosin.

### (*a.*) *Copper compounds.*

By adding either cupric sulphate or cupric acetate to a neutral ammonium chloride solution of myosin, a heavy greenish colored precipitate is obtained, which when freshly formed and after thorough washing with water, so that the washings are entirely free from chlorine and from copper, shows the following reactions. It is insoluble in moderately strong nitric, hydrochloric or sulphuric acid. The compound, however, is immediately broken up by the action of acids, the copper being completely removed, leaving the myosin as an insoluble residue having in the case of nitric acid a yellow color, and in the case of hydrochloric and sulphuric acids a white color. In acetic acid, the compound is more soluble, first, however, becoming semi-gelatinous. In ammonium hydroxide, the compound dissolves slowly or partially, taking on a blue color. In dilute sodium hydroxide, the compound swells up, takes on a purple color, but does not dissolve. In dilute sodium carbonate, the substance is likewise insoluble, but swells up and turns of a bluish color.

Following are the results of the analyses of the various preparations made. The compounds were in every case composed simply of the metallic oxide and myosin. Copper was determined, as in the case of the albumin compounds, by simple ignition and weighing as oxide. In order to ascertain how much ash was retained by the myosin compound, a few duplicate determinations of copper were made by dissolving the oxide after ignition, and precipitating the copper as sulphide and weighing as subsulphide, after ignition with a little pure sulphur in a current of hydrogen gas.

#### Series I.

*With* $CuSO_4$.

| No. | Amt. Sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu. |
|---|---|---|---|---|---|
| 1*a* | 0·5426 gram. | 0·0074 gram. | 1·08 | 0·0056 gram. | 0·81 |
| *b* | 0·7176 | 0·0097 | 1·07 | 0·0073 | 0·80 |
| 2*a* | 0·6359 | 0·0087 | 1·08 | 0·0071 | 0·88 |
| *b* | 0·5738 | 0·0078 | 1·08 | 0·0061 | 0·83 |

| No. | Amt. Sub. taken. | Wt. CuO. | Per cent. Cu. | Wt. $Cu_2S$. | Per cent. Cu. |
|---|---|---|---|---|---|
| 3*a* | 0·5423 gram. | 0·0072 gram. | 1·05 | 0·0051 gram. | 0·73 |
| *b* | 0·6241 | 0·0082 | 1·05 | 0·0063 | 0·80 |
| 4*a* | 0·5771 | 0·0109 | 1·50 | | |
| *b* | 0·4455 | 0·0081 | 1·43 | | |
| 5 | 0·3527 | 0·0046 | 1·02 | | |
| 6*a* | 0·4401 | 0·0062 | 1·11 | | |
| *b* | 0·5708 | 0·0082 | 1·13 | | |

## Series II.

### *With* $CuSO_4$.

| No. | Amt. Sub. taken. | Wt. CuO. | Per cent. CuO. | Per cent. Cu. |
|---|---|---|---|---|
| 1*a* | 0·7425 gram. | 0·0059 gram. | 0·79 | 0·63 |
| *b* | 0·7463 | 0·0060 | 0·81 | 0·64 |
| 2*a* | 0·7785 | 0·0057 | 0·73 | 0·57 |
| *b* | 0·7767 | 0·0056 | 0·72 | 0·58 |
| 3*a* | 0·5488 | 0·0052 | 0·94 | 0·74 |
| *b* | 0·4836 | 0·0041 | 0·94 | 0·73 |
| 4*a* | 0·9477 | 0·0085 | 0·89 | 0·74 |
| *b* | 0·8150 | 0·0074 | 0·91 | 0·73 |

### *With* $Cu(C_2H_3O_2)_2$.

| | | | | |
|---|---|---|---|---|
| 5*a* | 0·8522 | 0·0133 | 1·56 | 1·24 |
| *b* | 0·8466 | 0·0138 | 1·62 | 1·29 |
| 6*a* | 0·6589 | 0·0124 | 1·88 | 1·50 |
| *b* | 0·6572 | 0·0123 | 1·87 | 1·50 |
| 7*a* | 0·8673 | 0·0156 | 1·79 | 1·42 |
| *b* | 0·7120 | 0·0129 | 1·81 | 1·44 |

## Series III.

### *With* $CuSO_4$.

| No. | Amt. Sub. taken. | Wt. CuO. | Per cent. CuO. | Per cent. Cu. |
|---|---|---|---|---|
| 1*a* | 0·7213 gram. | 0·0171 gram. | 2·37 | 1·88 |
| *b* | 0·6584 | 0·0161 | 2·44 | 1·94 |
| 2*a* | 0·7564 | 0·0168 | 2·22 | 1·77 |
| *b* | 0·6817 | 0·0156 | 2·29 | 1·83 |
| 3*a* | 0·7820 | 0·0189 | 2·41 | 1·91 |
| *b* | 0·6213 | 0·0154 | 2·48 | 1·97 |
| 4*a* | 0·7137 | 0·0144 | 2·01 | 1·61 |
| *b* | 0·5863 | 0·0124 | 2·11 | 1·68 |
| 5*a* | 0·7732 | 0·0164 | 2·12 | 1·68 |
| *b* | 0·7407 | 0·0161 | 2·18 | 1·74 |
| 6*a* | 0·7447 | 0·0183 | 2·45 | 1·96 |
| *b* | 0·7429 | 0·0188 | 2·53 | 2·01 |

*With* $Cu(C_2H_3O_2)_2$.

| No. | Amt. Sub. taken. | Wt. CuO. | Per cent. CuO. | Per cent. Cu. |
|---|---|---|---|---|
| 7*a* | 0·7360 gram. | 0·0161 gram. | 2·18 | 1·73 |
| *b* | 0·7083 | 0·0154 | 2·17 | 1·73 |
| 8*a* | 0·7789 | 0·0174 | 2·24 | 1·78 |
| *b* | 0·8111 | 0·0181 | 2·23 | 1·77 |
| 9*a* | 0·7376 | 0·0169 | 2·29 | 1·81 |
| *b* | 0·6155 | 0·0138 | 2·24 | 1·78 |
| 10*a* | 0·7815 | 0·0191 | 2·44 | 1·94 |
| *b* | 0·8774 | 0·0213 | 2·43 | 1·93 |
| 11*a* | 0·8048 | 0·0170 | 2·11 | 1·67 |
| *b* | 0·8308 | 0·0174 | 2·10 | 1·67 |
| 12*a* | 0·6780 | 0·0150 | 2·21 | 1·75 |
| *b* | 0·7695 | 0·0168 | 2·18 | 1·74 |

Comparing these results with one another, there is to be seen a very noticeable lack of agreement in composition, and further it is to be seen that the myosin-copper compound has on an average a somewhat higher content of copper than the albumin-copper precipitate. The average composition of all the copper myosins shows about 1·42 per cent. of Cu, and deducting 0·25 per cent. of ash, the average content of Cu would be 1·17 per cent. Examining the individual results, it is apparent that the compounds made from the same myosin solution are approximately, at least, the same in composition and without doubt the difference in the composition of compounds made from different myosin solutions is due to variation in the concentration of, and possibly also in the reaction of, the myosin-containing fluid. It would appear as if variations in the conditions of precipitation made a greater difference in the case of the myosin-copper compounds than in the compounds of copper with albumin. Several times, also, we have found that our myosin-copper precipitate contained sulphuric acid, even after thorough washing and when the wash-water was proved to be entirely free from any reaction with barium chloride.

One such compound, after drying at 110° C., was analyzed with the following results:

0·7240 gram substance* gave 0·0343 gram $BaSO_4$ = 1·63 per cent. $SO_3$.
0·8420 gram substance gave 0·0331 gram $BaSO_4$ = 1·73 per cent. $SO_3$.
0·4705 gram substance† gave 0·0084 gram CuO = 1·78 per cent. CuO.
0·6011 gram substance gave 0·0107 gram CuO = 1·78 per cent. CuO.

* Roasted and then ignited with pure sodium carbonate, the residue dissolved in hot water acidified with hydrochloric acid and precipitated with barium chloride.

† Ignited, the residue dissolved in nitric acid, precipitated with hydrogen sulphide, etc.

The molecular weight of CuO and $SO_3$ being the same, it is evident that the two are present in just the proportion to form cupric sulphate.

### *b. Iron compounds.*

By adding a solution of ferric chloride to an ammonium chloride solution of myosin, a semi-gelatinous precipitate is formed of a reddish yellow color, and consisting of a combination of myosin and oxide of iron. The compound when thoroughly washed contains no chlorine. When freshly precipitated, it is partially soluble in dilute ammonium hydroxide, as also in sodium hydroxide, the residue becoming gummy or gelatinous and brownish yellow in color. It swells up in sodium carbonate, but is insoluble. In nitric acid the compound turns yellow, but is wholly insoluble and does not swell up. In hydrochloric and also in sulphuric acid the compound is likewise insoluble. In acetic acid, however, it is soluble completely, forming a semi-gelatinous fluid. In this, as in other metallic compounds of myosin, acids simply dissolve out the metal and then exert their usual action on the myosin. The various preparations, washed free from iron and chlorine, and dried at 110° C. were analyzed with the following results:

SERIES I.

| No. | Amt. Sub. taken. | Wt. $Fe_2O_3$. | Per cent. $Fe_2O_3$. | Per cent. Fe. |
|---|---|---|---|---|
| 1*a* | 0·7046 gram. | 0.0194 gram. | 2·76 | 1·93 |
| *a* | 0·7021 | 0·0193 | 2·74 | 1·92 |
| 2*a* | 0·3762 | 0·0128 | 3·40 | 2·38 |
| *b* | 0·3623 | 0·0121 | 3·33 | 2·33 |
| 3*a* | 0·5837 | 0·0141 | 2·42 | 1·69 |
| *b* | 0·5618 | 0·0137 | 2·43 | 1·70 |
| 4*a* | 0·5438 | 0·0177 | 3·25 | 2·26 |
| *b* | 0·6088 | 0·0197 | 3·24 | 2·26 |
| 5*a* | 0·4761 | 0·0140 | 2·95 | 2·06 |
| *b* | 0·3381 | 0·0100 | 2·95 | 2·07 |
| 6*a* | 0·5101 | 0·0173 | 3·40 | 2·37 |
| *b* | 0·5927 | 0·0194 | 3·28 | 2·29 |

SERIES II.

| | | | | |
|---|---|---|---|---|
| 1 | 0·4847 | 0·0188 | 3·87 | 2·70 |
| 2 | 0·3200 | 0·0115 | 3·59 | 2·51 |
| 3 | 0·4118 | 0·0153 | 3·72 | 2·59 |
| 4 | 0.5205 | 0·0184 | 3·53 | 2·45 |

| No. | Amt. Sub. taken. | Wt. $Fe_2O_3$. | Per cent. $Fe_2O_3$. | Per cent. Fe. |
|---|---|---|---|---|
| 5*a* | 0·4551 gram. | 0·0167 gram. | 3·68 | 2·57 |
| *b* | 0·3668 | 0·0137 | 3·73 | 2·61 |
| 6*a* | 0·3783 | 0·0138 | 3·64 | 2·53 |
| *b* | 0·3675 | 0·0131 | 3·57 | 2·50 |
| 7*a* | 0·4284 | 0·0143 | 3·34 | 2·33 |
| *b* | 0·4041 | 0·0136 | 3·37 | 2·36 |

SERIES III.

*With a large excess of ferric chloride.*

| | | | | |
|---|---|---|---|---|
| 1*a* | 0·4385 | 0·0269 | 6·13 | 4·29 |
| *b* | 0·6939 | 0·0436 | 6·27 | 4·38 |

In none of these preparations was there any attempt made to add a definite amount of ferric chloride, but the iron salt was added until a good precipitate was obtained. Undoubtedly, the amount of iron salt added, modifies materially the composition of the compound. In series III it is seen that the content of iron is about double the average amount contained in the other preparations. The average amount of iron (Fe) in the first two series of compounds is 2·29 per cent.

### *c. Zinc compounds.*

With zinc sulphate, myosin is thrown down from its ammonium chloride solution as a heavy gelatinous precipitate. Like the iron compound it is partially soluble in sodium and ammonium hydroxides, swelling up to a gelatinous mass. It is insoluble in nitric, hydrochloric and sulphuric acids, but is partially soluble in acetic acid. In composition, it is seen to be very closely allied to the zinc albuminate. Following are the results obtained by analysis of the dried compounds:

SERIES I.

| No. | Amt. Sub. taken. | Wt. ZnO. | Per cent. ZnO. | Per cent. Zn. |
|---|---|---|---|---|
| 1*a* | 0·6108 gram. | 0·0049 gram. | 0·81 | 0·64 |
| *b* | 0·6619 | 0·0055 | 0·83 | 0·66 |
| 2*a* | 0·6611 | 0·0047 | 0·71 | 0·57 |
| *b* | 0·8006 | 0·0059 | 0·73 | 0·59 |
| 3*a* | 0·4666 | 0·0046 | 0·99 | 0·79 |
| *b* | 0·4494 | 0·0044 | 0·99 | 0·79 |
| 4*a* | 0·5936 | 0·0048 | 0·80 | 0·64 |
| *b* | 0·6926 | 0·0055 | 0·79 | 0·63 |

SERIES II.

| No. | Amt. Sub. taken. | Wt. ZnO. | Per cent. ZnO. | Per cent. Zn. |
|---|---|---|---|---|
| 1*a* | 0·6258 gram. | 0·0051 gram. | 0·82 | 0·65 |
| *b* | 0·6618 | 0·0054 | 0·81 | 0·65 |
| 2*a* | 0·5315 | 0·0064 | 1·21 | 0·97 |
| *b* | 0·5485 | 0·0065 | 1·18 | 0·94 |
| 3*a* | 0·7925 | 0·0064 | 0·81 | 0·65 |
| *b* | 0·6913 | 0·0057 | 0·83 | 0·66 |
| 4*a* | 0·4858 | 0·0062 | 1·27 | 1·02 |
| *b* | 0·5318 | 0·0066 | 1·24 | 0·99 |
| 5*a* | 0·6635 | 0·0056 | 0·85 | 0·68 |
| *b* | 0·6504 | 0·0050 | 0·76 | 0·61 |
| 6*a* | 0·6263 | 0·0055 | 0·87 | 0·70 |
| *b* | 0·6639 | 0·0059 | 0·88 | 0·71 |

The average content of zinc (Zn) is 0·72 per cent. Unlike the iron and copper compounds, there is here less variation in the composition of the various preparations.

### *d. Nickel and cobalt compounds.*

The extremely low percentage of zinc in the zinc-myosin compounds, as contrasted with the iron in the iron compounds, led us to make a nickel and cobalt preparation for the sake of comparison. The results, in both cases, accord more nearly with those of the iron compound, for although containing a higher percentage of metal than the latter, it was necessary to prepare them both under just such conditions as in the iron compound led to the highest percentage of iron, viz: a large excess of the precipitant. Following are the analytical results obtained with both substances:

*With* Ni $(NO_3)_2$.

| No. | Amt. Sub. taken. | Wt. $NiO_2$. | Per cent. $NiO_2$. | Per cent. Ni. |
|---|---|---|---|---|
| 1*a* | 0·6165 gram. | 0·0045 gram. | 7·29 | 4·71 |
| *b* | 0·7016 | 0·0051 | 7·26 | 4·70 |

*With* CO $(NO_3)_2$.

| | | | | |
|---|---|---|---|---|
| 1*a* | 0·6479 | 0·0064 | 9·95 | 6·45 |
| *b* | 0·6696 | 0·0065 | 9·70 | 6·28 |
| 2*a* | 0·6451 | 0·0056 | 8·75 | 5·67 |
| *b* | 0·6842 | 0·0060 | 8·84 | 5·72 |

### *e. Uranium compounds.*

The addition of uranyl nitrate to an ammonium chloride solution of myosin produces a heavy gelatinous precipitate of a uranyl-myosin compound, which in solubility resembles the other myosin preparations. Washed free from excess of uranyl nitrate and from ammonium chloride and then dried at 110° C., the various preparations yielded on analysis the following results, the uranium being determined by simple ignition and weighing as uranoso-uranic oxide:

SERIES I.

| No. | Amt. Sub taken. | Wt. $U_3O_8$. | Per cent. $U_3O_8$. | Per cent. U. |
|---|---|---|---|---|
| 1*a* | 0·6378 gram. | 0·0489 gram. | 7·67 | 6·51 |
| *b* | 0·6836 | 0·0526 | 7·69 | 6·53 |
| 2*a* | 0·7081 | 0·0592 | 8·36 | 7·09 |
| *b* | 0·7689 | 0·0655 | 8·51 | 7·22 |
| 3*a* | 0·6418 | 0·0586 | 9·13 | 7·75 |
| *b* | 0·7809 | 0·0715 | 9·16 | 7·78 |
| 4*a* | 0·6621 | 0·0541 | 8·17 | 6·93 |
| *b* | 0·7455 | 0·0608 | 8·15 | 6·91 |
| 5*a* | 0·7525 | 0·0663 | 8·81 | 7·48 |
| *b* | 0·6964 | 0·0615 | 8·83 | 7·50 |
| 6*a* | 0·7208 | 0·0590 | 8·18 | 6·94 |
| *b* | 0·7439 | 0·0607 | 8·15 | 6·91 |
| 7*a* | 0·6648 | 0·0527 | 7·92 | 6·72 |
| *b* | 0·7101 | 0·0565 | 7·96 | 6·76 |

SERIES II.

| | | | | |
|---|---|---|---|---|
| 1*a* | 0·8736 | 0·0837 | 9·58 | 8·13 |
| *b* | 0·8570 | 0·0820 | 9·57 | 8·12 |
| 2*a* | 0·5857 | 0·0593 | 10·12 | 8·59 |
| *b* | 0·7088 | 0·0715 | 10·09 | 8·56 |
| 3*a* | 0·5715 | 0·0568 | 9·94 | 8·44 |
| *b* | 0·6958 | 0·0688 | 9·89 | 8·40 |
| 4*a* | 0·6929 | 0.0556 | 8·02 | 6·82 |
| *b* | 0·7667 | 0·0615 | 8·02 | 6·82 |
| 5*a* | 0·8031 | 0·0874 | 10·88 | 9·23 |
| *b* | 0·8272 | 0·0901 | 10·89 | 9·24 |
| 6*a* | 0·7034 | 0·0553 | 7·86 | 6·67 |
| *b* | 0·6810 | 0·0540 | 7·92 | 6·72 |

These results show a variation in the content of uranium, amounting to nearly 3 per cent. (6·51–9·24 per cent.) Further, a compari-

son of the two series shows plainly that there is something in the nature of the second myosin solution, which tends to raise the content of uranium in the uranyl compounds; probably, the greater concentration or dilution of the solution. Evidently, then, the composition of the compound is, in part at least, determined by the conditions under which the uranyl salt and the myosin solution are brought together. The average amount of uranium contained in the preparations is 7·49 per cent.

### *f. Mercury compounds.*

By adding a solution of mercuric chloride to an ammonium chloride solution of myosin, a heavy gelatinous precipitate is formed which soon changes to a flocculent one. Freed from the excess of mercury salt and ammonium chloride, the compound is found to be entirely free from chlorine. The substance is somewhat soluble in sodium hydroxide, swelling up first and then gradually dissolving. Dried at 110° C. and then analyzed, the following results were obtained. The mercury was determined as already described under mercury albuminate.

SERIES I.

| No. | Amt. Sub. taken. | Wt. of Hg. | Per cent. Hg. |
|---|---|---|---|
| 1*a* | 0·7609 gram. | 0·0166 gram. | 2·18 |
| *b* | 1·2911 | 0·0268 | 2·07 |
| 2*a* | 1·2386 | 0·0270 | 2·17 |
| *b* | 0·9525 | 0·0198 | 2·07 |
| 3*a* | 1·1856 | 0·0218 | 1·84 |
| *b* | 0·9261 | 0·0173 | 1·86 |
| 4*a* | 1·3504 | 0·0257 | 1·90 |
| *b* | 0·9332 | 0·0178 | 1·90 |

SERIES II.

| | | | |
|---|---|---|---|
| 1 | 0·8917 | 0·0241 | 2·70 |
| 2 | 1·1196 | 0·0310 | 2·77 |
| 3*a* | 0·9537 | 0·0271 | 2·84 |
| *b* | 0·8813 | 0·0259 | 2·93 |
| 4 | 0·9209 | 0·0270 | 2·93 |
| 5 | 0·8352 | 0·0241 | 2·89 |
| 6 | 0·8564 | 0·0217 | 2·53 |
| 7*a* | 1·0696 | 0·0344 | 3·22 |
| *b* | 0·8550 | 0·0264 | 3·09 |

The average content of mercury (Hg) is 2·43 per cent. The results of each series show a fairly close agreement, but the two series do not compare with each other at all. Thus, the average amount of mercury in the compounds of the first series is 1·99 per cent., while in the second series the average amount rises to 2·87 per cent. This is another good illustration of the influence of the strength of the solution on the composition of the precipitate, and as in this case the presence of any ash could not interfere with the ultimate result, since the mercury was separated by distillation, it follows that the apparently higher content of mercury in the second series must be due to combination of the myosin with a larger amount of the metal. Further, it has been claimed* that in the case of the silver albuminate, it is possible under certain circumstances for the albuminate, when formed in a concentrated solution, to inclose a variable amount of albumin mechanically, and thus the apparent percentage of silver in the albuminate be reduced. If such was true of the myosin-mercury compounds, a far greater variation would be expected in the percentage of mercury in the different preparations of the same series.

The following table of comparisons shows the average content of metal in the albuminates formed from the two kinds of proteid matter.

| | | Egg-albumin. | | Myosin. | |
|---|---|---|---|---|---|
| Copper compound, | | 0·94 per cent. | Cu | 1·17 per cent. | Cu |
| Iron | " | 0·95 | Fe | 2·29 | Fe |
| Zinc | " | 0·91 | Zn | 0·72 | Zn |
| Uranyl | " | 4·60 | U | 7·49 | U |
| Mercury | " | 2·89 | Hg | 2·43 | Hg |
| Lead | " | 2·56 | Pb | ---- | ---- |
| Silver | " | 4·09 | Ag | ---- | ---- |
| Nickel | " | | | 4·70 | Ni |
| Cobalt | " | | | 6·03 | Co |

Apparently, the two forms of albuminous matter, the albumin and globulin, do not form corresponding compounds with the metallic salts experimented with.

* See Loew, Pflüger's Archiv für Physiologie, Band xxxi, p. 393.

## XXI.—Egg-Albumin and Albumoses. By R. H. Chittenden and Percy R. Bolton, Ph.B.

Ever since the albumose bodies were first separated from the products of fibrin digestion* with pepsin-hydrochloric acid, it has been our intention, already expressed, to subject the various individual albumins† to the action of purified pepsin under like conditions, and thus ultimately to acquire a comparative knowledge of the albumose bodies obtainable from these different sources. Already the albumose bodies from fibrin and the globuloses‡ have been subjected to a careful study and we present here the result of a study of the albumoses from egg-albumin.

In doing this, we have to report at the same time, the results of a study of the composition of egg-albumin itself. For we have made it a rule, in the series of experiments shortly to be described, to analyze a sample of each lot of albumin prepared for digestion. In this manner we have obtained data for a direct comparison of composition between the original sample of albumin and the products formed by its digestion. This we have deemed of considerable importance, for the data so obtained may throw considerable light on the nature of the changes involved in the formation of the albumoses; particularly, as to whether they are hydrolytic in their nature.

Four distinct samples of albumin were prepared, three of which were prepared in large quantities and served as material for the subsequent digestions.

### *Albumin A.*

This was a preparation of coagulated egg-albumin, prepared especially with the view of obtaining a product wholly free from globulin. The method employed was essentially that recommended by Hammarsten.§ The whites of 120 eggs were freed from the yolks, then

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* W. Kühne and R. H. Chittenden, Ueber Albumosen, Zeitschrift für Biologie, Band xx, p. 11.

† W. Kühne and R. H. Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskörper, Zeitschrift für Biologie, Band xix, p. 159.

‡ W. Kühne and R. H. Chittenden, Globulin und Globulosen, Zeitschrift für Biologie, Band xxii, p. 409.

§ See K. V. Starke, Beiträge zur Kenntniss des Serum- und Eialbumins, Jahresbericht für Thierchemie, 1881, p. 18.

finely divided by shaking with glass, the fluid mixed with an equal volume of water or more, then shaken vigorously with air and finally filtered through cloth. The solution so obtained, was then saturated with crystals of magnesium sulphate at 20° C., for the complete removal of the globulin. The mixture was filtered through paper and the clear filtrate saturated with sodium sulphate. The precipitated albumin was then filtered and washed with a saturated solution of sodium sulphate, after which it was dissolved in water and dialyzed in running water until the magnesium and sodium sulphates were entirely removed. The fluid was then again filtered and the albumin finally coagulated by being poured into eight litres of boiling water, slightly acidified with acetic acid. The great bulk of the coagulum so obtained was at once placed in four litres of 0·4 per cent. hydrochloric acid, while a small sample for analysis was washed with 95 per cent. alcohol, finally with absolute alcohol and then dried, first at 100° C., and finally at 106° C., *in vacuo*, until of constant weight. The following table shows the results of the analysis of the product. The various determinations were made as described in the previous articles on these subjects, the sulphur being determined by fusion with potassium hydroxide and potassium nitrate in a silver crucible, according to the method designated by Hammarsten* as 1*a*.

### *Albumin B.*

This albumin was prepared from the whites of 120 eggs by a somewhat different method. The albumin solution, after dilution with water, was made very distinctly acid with acetic acid, and the heavy precipitate of globulin, after it had well settled, removed by filtration. The acid fluid was then made exactly neutral with sodium carbonate and again filtered; it was then thymolized and dialyzed in running water for eight days. A little globulin, not precipitated by the acetic acid, was found in the bottom of the dialyzers when the salts had diffused out. This was filtered off and the perfectly clear fluid evaporated at 35–45° C., to perfect dryness. A sample of this preparation was ground fine, dried at 106° C. *in vacuo* and analyzed. It is perhaps questionable, whether all of the globulin is removed by this method. The precipitate with acetic acid was quite heavy, and as H. Dillner† has recently shown that the amount of globulin in egg-albumin, as

* See Zeitschrift für physiolog. Chemie, Band ix, p. 289.

† Ueber die Globuline im Hühnereiweiss, Jahresbericht für Thierchemie, 1885, p. 31.

ANALYSIS OF ALBUMIN A.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3938 | 0·2455 | 6·92 | 0·7517 | 52.05 | .... | ... | ..... | ..... | ..... | .... | ..... | .... |
| II | 0·6406 | 0·4007 | 6·95 | 1·2247 | 51.98 | .... | ... | ..... | .... | ..... | .... | ..... | .... |
| III | 0·6133 | ..... | .... | ..... | .... | 80·5 | 8·2 | 749·1 | 15·78 | ..... | .... | ..... | .... |
| IV | 0·6189 | ..... | .... | ..... | .... | 79·8 | 6·8 | 754·5 | 15·68 | ..... | .... | ..... | .... |
| V | 0·5209 | ..... | .... | ..... | .... | 66·6 | 6·8 | 766·9 | 15·81 | ..... | .... | ..... | .... |
| VI | 0·5751 | ..... | .... | ..... | .... | .... | ... | ..... | ..... | 0·0798 | 1·90 | ..... | .... |
| VII | 0·5926 | ..... | .... | ..... | .... | .... | ... | ..... | ..... | 0·0833 | 1·93 | ..... | .... |
| VIII | 0·6417 | ..... | .... | ..... | .... | .... | ... | ..... | ..... | 0·0916 | 1·96 | ..... | .... |
| IX | 0·6366 | ..... | .... | ..... | .... | .... | ... | ..... | ..... | ..... | .... | 0·0023 | 0·37 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|
| C | 52·24 | 52·18 | .... | .... | .... | .... | .... | .... | *52·21* |
| H | 6·95 | 6·98 | .... | .... | .... | .... | .... | .... | *6·96* |
| N | .... | .... | 15·83 | 15·74 | 15·85 | .... | .... | .... | *15·80* |
| S | .... | .... | .... | .... | .... | 1·91 | 1·94 | 1.97 | *1·94* |
| O | .... | .... | .... | .... | .... | .... | .... | .... | *23·09* |
| | | | | | | | | | *100·00* |

ANALYSIS OF ALBUMIN B.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5525 | 0·3447 | 6·98 | 1·0483 | 51·70 | .... | --- | ----- | .... | ------ | .... | ------ | .... |
| II | 0·6350 | 0·3944 | 6·90 | 1·2070 | 51·83 | .... | .... | ----- | .... | ------ | .... | ------ | .... |
| III | 0·4499 | ----- | .... | ----- | .... | 57·6 | 9·6 | 768·1 | 15·70 | ------ | .... | ------ | .... |
| IV | 0·3088 | ----- | .... | ----- | .... | 41·4 | 15·6 | 751·0 | 15·74 | ------ | .... | ------ | .... |
| V | 0·6240 | ----- | .... | ----- | .... | .... | .... | ----- | .... | 0·0796 | 1·75 | ----- | .... |
| VI | 0·6166 | ----- | .... | ----- | .... | .... | .... | ----- | .... | 0·0856 | 1·90 | ------ | .... |
| VII | 0·5464 | ----- | .... | ----- | .... | .... | .... | ----- | .... | 0·0688 | 1·71 | ------ | .... |
| VIII | 0·5040 | ----- | .... | ----- | .... | .... | --- | ----- | .... | 0·0670 | 1·82 | ------ | .... |
| IX | 0·5690 | ----- | .... | ----- | .... | .... | .... | ----- | .... | 0·0751 | 1·81 | ------ | .... |
| X | 0·4430 | ----- | .... | ----- | .... | --- | .... | ----- | .... | ------ | .... | 0·0049 | 1·10 |
| XI | 0·4901 | ----- | .... | ----- | .... | .... | .... | ----- | .... | ------ | .... | 0·0055 | 1·12 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|---|
| C | 52·28 | 52·39 | .... | .... | .... | --- | .... | .... | .... | *52·33* |
| H | 7·00 | 6·97 | .... | .... | .... | .... | .... | .... | .... | *6·98* |
| N | .... | .... | 15·87 | 15·91 | .... | --- | .... | .... | .... | *15·89* |
| S | .... | .... | .... | .... | 1·78 | 1·93 | 1·74 | 1·85 | 1·84 | *1·83* |
| O | .... | .... | .... | .... | .... | .... | .... | .... | .... | *22·97* |
| | | | | | | | | | | *100·00* |

determined by the magnesium sulphate method, never reaches 1 per cent., but averages only 0·667 per cent., it seems probable that the greater portion is separated by the acetic acid. Further, Dillner has found that on the dialysis of a neutralized egg-albumin solution, the matter which separates out after a few days dialysis, is only in part globulin, but consists, in addition, of a somewhat insoluble body rich in sulphur. Hence, the substance which separated in our dialyzers, after precipitation with acetic acid and neutralization, may not have been composed wholly of globulin. The following table shows the composition of the uncoagulated albumin B.

### *Albumin C.*

This preparation was much the same as albumin B, except that it was finally coagulated. Globulin was separated by acetic acid, the filtrate neutralized, again filtered and the fluid dialyzed in running water until all soluble salts were removed. The albumin was then coagulated by being poured into a large volume of boiling water acidified with acetic acid. A sample, after drying at 106° C. *in vacuo*, was found to have the composition shown in the accompanying table.

### *Albumin D.*

This sample of albumin was prepared in exactly the same manner as albumin A; the globulin removed by magnesium sulphate, the albumin precipitated by sodium sulphate and after dialysis, coagulated as already described. Its composition is shown in the following table.

Comparing now, the results of the analysis of these four samples of albumin, it is seen that the first three agree almost exactly in composition, while the fourth shows a somewhat lower content of carbon.

| | *A* | *B* | *C* | *D* | *Average.* |
|---|---|---|---|---|---|
| C | 52·21 | 52·33 | 52·46 | 51·74 | 52·18 |
| H | 6·96 | 6·98 | 7·00 | 6·81 | 6·93 |
| N | 15·80 | 15·89 | 15·88 | 15·68 | 15·81 |
| S | 1·94 | 1·83 | 1·69 | 2·02 | 1·87 |
| O | 23·09 | 22·97 | 22·97 | 23·75 | 23·21 |
| Ash | 0·37 | 1·11 | 0·17 | 0·45 | |

Further, the coagulated products (A and C) do not differ at all in composition from the non-coagulated albumin B.

Schützenberger,* as a result of his work on proteid matter, ascribed

* Bulletin de la Société Chimique de Paris, T. 23 et 24.

ANALYSIS OF ALBUMIN C.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. ° C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·2930 | 0·1850 | 7·01 | 0·5633 | 52·42 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·6268 | 0·3933 | 6·97 | 1·2033 | 52·35 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5936 | ---- | ---- | ---- | ---- | 79·5 | 15·7 | 752·4 | 15·74 | ---- | ---- | ---- | ---- |
| IV | 0·3742 | ---- | ---- | ---- | ---- | 50·8 | 16·3 | 754·8 | 15·98 | ---- | ---- | ---- | ---- |
| V | 0·5979 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0736 | 1·69 | ---- | ---- |
| VI | 0·7139 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0872 | 1·68 | ---- | ---- |
| VII | 0·7333 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0013 | 0·17 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·49 | 52·43 | ---- | ---- | ---- | ---- | *52·46* |
| H | 7·02 | 6·98 | ---- | ---- | ---- | ---- | *7·00* |
| N | ---- | ---- | 15·77 | 16·00 | ---- | ---- | *15·88* |
| S | ---- | ---- | ---- | ---- | 1·69 | 1·68 | *1·69* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·97* |
| | | | | | | | *100·00* |

ANALYSIS OF ALBUMIN D.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·6009 | 0·3695 | 6·83 | 1·1340 | 51·46 | ---- | ---- | ----- | ---- | ----- | ---- | ----- | ----- |
| II | 0·4564 | 0·2765 | 6·73 | 0·8630 | 51·56 | ---- | ---- | ----- | ---- | ----- | ---- | ----- | ----- |
| III | 0·5094 | ----- | ---- | ----- | ---- | 65·1 | 6·0 | 756·5 | 15·68 | ----- | ---- | ----- | ----- |
| IV | 0·7652 | ----- | ---- | ----- | ---- | 96·0 | 4·7 | 765·5 | 15·60 | ----- | ---- | ----- | ----- |
| V | 0·9402 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | 0·1379 | 2·01 | ----- | ----- |
| VI | 0·6664 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | 0·0982 | 2·02 | ----- | ----- |
| VII | 1·2907 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | ----- | ---- | 0·0058 | 0·45 |
| VIII | 1·0975 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | ----- | ---- | 0·0051 | 0·46 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·69 | 51·79 | ---- | ---- | ---- | ---- | *51·74* |
| H | 6·86 | 6·76 | ---- | ---- | ---- | ---- | *6·81* |
| N | ---- | ---- | 15·69 | 15·67 | ---- | ---- | *15·68* |
| S | ---- | ---- | ---- | ---- | 2·02 | 2·08 | *2·02* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *23·75* |
| | | | | | | | *100·00* |

to albumin the formula $C_{240}H_{392}N_{66}O_{75}S_3$, which requires a content of carbon not far different from the average of our results, but which on the other hand demands a content of nitrogen nearly 1 per cent. higher than we found. The well-known Lieberkühn's formula requires 53·59 per cent. of carbon, or 1 per cent. more than was found in our highest result. Harnack's formula for albumin,* $C_{204}H_{322}N_{52}O_{66}S_2$, with a molecular weight of 4618, based on a study of the copper compounds of albumin, requires too high a content of carbon and altogether too low a percentage of sulphur. Lieberkühn's formula requires 1·98 per cent. of sulphur, while Harnack's formula requires only 1·39 per cent.; and as this was one of the main points on which Harnack based his formula, it is well to consider it. Our lowest result on sulphur is 1·69 per cent., and as the other three show a close agreement, it is probable that the former is somewhat too low. The average of our results, however, is but 0·04 per cent. higher than found by Lieberkühn. O. Loew† has recently considered this question, and he found on determining the sulphur in coagulated egg-albumin by a modification of Piria and Schiff's method, 1·70 and 1·87 per cent. of sulphur respectively. O. Nasse,‡ likewise, found in coagulated albumin a content of 1·72 per cent. of sulphur, and lastly, Hammarsten§ found in non-coagulated albumin 1·93 per cent. of sulphur. There would seem to be plenty of confirmatory evidence, therefore, that the content of sulphur in egg-albumin is much larger than indicated by Harnack's formula.

The nitrogen, as determined in our preparations, is seen to be somewhat higher than found by Hammarsten, with whose results in other respects ours most closely correspond. Dumas, however, found nearly the same percentage of nitrogen as contained in our preparations. The accompanying table of analyses shows the average of our results, compared with those of others.

### *Albumoses.*

Three distinct digestions of albumin (preparations A, B and C) were made with pepsin-hydrochloric acid, and the albumose bodies isolated. In this way it was possible to prepare the bodies under somewhat different conditions, and to notice the influence, if any, on the nature and composition of the products. The pepsin-hydrochloric acid used in two of the digestions was prepared with a special view

* Zeitschrift für Physiolog. Chemie, Band v, p. 207.

† Pflüger's Archiv für Physiologie, Band xxxi, p. 395.

‡ Jahresbericht für Thierchemie, 1873, p. 13. § Ibid, 1881, p. 19

## Analyses of Egg-Albumin.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| C | 53·5 | 53·51 | 53·87 | 52·25 | 52·18 | 53·59 | 52·58 | 53·01 |
| H | 7·0 | 7·08 | 7·10 | 6·90 | 6·98 | 6·95 | 7·15 | 6·98 |
| N | 15·5 | 15·61 | 15·77 | 15·25 | 15·81 | 15·65 | 16·61 | 15·76 |
| S | 1·6 | 1·83 | ----- | 1·98 | 1·87 | 1 98 | 1·75 | 1·39 |
| O | ---- | ----- | ----- | ----- | ---- | 21·88 | 21·91 | 22·86 |

1. As analyzed by Mulder, Journal für prakt. Chemie, Band xliv, p. 509.
2. As analyzed by Lieberkühn. Poggendorff's Annalen, 1852, p. 117.
3. As analyzed by Dumas and Cahous. Ann. chem. Phys. (3) vi, p. 408.
4. As analyzed by Hammarsten. Jahresbericht für Thierchemie, 1881, p. 19.
5. Average of the results by Chittenden and Bolton.
6. Theoretical composition according to Lieberkühn's formula ($C_{72}H_{112}N_{18}SO_{22}$).
7. Theoretical composition according to Schützenberger's formula ($C_{240}H_{382}N_{64}O_{75}S_2$)
8. Theoretical composition according to Harnack's formula ($C_{204}H_{322}N_{52}O_{66}S_2$).

to removing all traces of albumose bodies, formed by the self-digestion of the mucous membrane, and was prepared as follows: 700 grams of mucous membrane from the cardiac portion of six pigs' stomachs, freed from the muscularis, were finely divided and warmed at 40° C. for fourteen days, in two and a half litres of 0·5 per cent. hydrochloric acid. At the end of this time, all albumose bodies presumably having been converted into peptone, the solution was filtered from the residue of nuclein, antialbumid, etc., and the filtrate saturated with ammonium sulphate. The precipitate, consisting mainly of pepsin, with perhaps some albumose, was filtered off, washed with a saturated solution of ammonium sulphate, and then dissolved in two litres of 0·2 per cent. hydrochloric acid. The acid solution was then thymolized and dialyzed in running water, until the ammonium sulphate was entirely removed. On opening the dialyzing tubes, quite a precipitate was found, which on being dissolved in 0·2 per cent. hydrochloric acid showed marked proteolytic action. The filtrate also, on being acidified, showed vigorous digestive power. These two solutions of purified pepsin were used in the digestion of two of the albumins, while with the third a pure glycerin extract of pepsin was employed.

The general method of procedure, both in the digestions themselves and in the separation of the various albumoses, was much the same as that previously employed by Kühne and Chittenden.

### *Digestion of Albumin A.*

The albumin, as previously described, was placed in four litres of 0·4 per cent. hydrochloric acid and the mixture raised to a temperature of 45° C. Then 600 c. c. of the purified pepsin-hydrochloric acid solution were added and the mixture kept at a temperature of 45° C. for three hours, after which it was neutralized with sodium hydroxide and filtered. The pepsin solution, although quite active, did not act very vigorously on the coagulated albumin. The neutralization precipitate, therefore, together with the unaltered albumin, was again treated with a fresh quantity of the pepsin-hydrochloric acid, under like conditions as the preceding, for four hours. The two neutralized fluids were then united and treated together. The total volume was about six litres. The clear fluid was saturated in the cold with crystals of sodium chloride, by which a precipitate was obtained, which from analogy should consist of proto-, dys- and heteroalbumose.

In making these separations of the albumose bodies, we intentionally avoided raising the temperature of the fluid above 45° C., for fear that heat might induce some change in the character of the bodies; hence the first neutralized fluid was saturated directly with salt, in spite of its large volume, and the bodies were ultimately all separated without having been exposed to a temperature higher than that above-mentioned. The use, however, of such a large quantity of rock salt introduced into the solutions some calcium sulphate, which adhered very tenaciously to the albumose bodies and thus unavoidably raised the content of ash in the preparations.

The precipitate produced by the addition of sodium chloride in substance was filtered, washed with a saturated solution of sodium chloride, then extracted successively with a ten per cent. solution of sodium chloride, a five per cent. solution of the same salt, and lastly with water. The residue remaining undissolved after these successive treatments with dilute salt solutions and water, presumably consisted of dysalbumose, while the solutions contained a body precipitable by acetic acid and soluble in excess, and also precipitable by potassium ferrocyanide; presumably protoalbumose together with heteroalbumose. The original salt-saturated filtrate contained all of the deuteroalbumose, together with considerable protoalbumose and some heteroalbumose.

### *A. Protoalbumose.*

The five and ten per cent. sodium chloride solutions of the first salt precipitate, together with the aqueous solution of the same, were united and then dialyzed in running water for removal of the heteroalbumose. The solution, partially freed from the latter, was concentrated somewhat and the protoalbumose again precipitated by saturating the solution with sodium chloride. This precipitate was again dissolved in water, dialyzed until the greater portion of the salt was removed, the solution then concentrated and the albumose precipitated by alcohol. This precipitate was redissolved in water, dialyzed until no chlorine reaction could be obtained with silver nitrate, the solution concentrated on the water bath to a syrup and finally precipitated with alcohol, washed with alcohol and ether and then dried at 106° C. *in vacuo* until of constant weight. In the last dialysis, there was no separation whatever of heteroalbumose, hence the protoalbumose is to be considered as quite pure. The composition of the substance is shown in the accompanying table. The ash contained no sulphate.

ANALYSIS OF A. PROTOALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4428 | 0·2699 | 6·77 | 0·8062 | 49·65 | .... | .... | ..... | .... | ..... | .... | ..... | .... |
| II | 0·2665 | 0·1607 | 6·70 | 0·4862 | 49·75 | .... | .... | ..... | .... | ..... | .... | ..... | .... |
| III | 0·4825 | ..... | .... | ..... | .... | 63·0 | 15·2 | 753·4 | 15·40 | ..... | .... | ..... | .... |
| IV | 0·4038 | ..... | .... | ..... | .... | 52·1 | 14·3 | 758·2 | 15·36 | ..... | ... | ..... | .... |
| V | 0·5392 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0673 | 1·71 | ..... | .... |
| VI | 0·5516 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0733 | 1·80 | ..... | .... |
| VII | 0·6856 | ..... | .... | ..... | .... | .... | .... | ..... | .... | ..... | .... | 0·0172 | 2·50 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 50·92 | 51·03 | .... | .... | .... | .... | *50·98* |
| H | 6·94 | 6·87 | .... | .... | .... | .... | *6·90* |
| N | .... | .... | 15·79 | 15·75 | .... | .... | *15·77* |
| S | .... | .... | .... | .... | 1·75 | 1·86 | *1·81* |
| O | .... | .... | .... | .... | .... | .... | *24·54* |
| | | | | | | | *100·00* |

The protoalbumose was readily soluble in water and, unlike the protoalbumose from fibrin, dissolved to a perfectly clear solution with a neutral or very faintly alkaline reaction. The aqueous solution was rendered somewhat turbid by the addition of a little acetic acid, the turbidity disappearing, however, on the addition of an excess of acid. The aqueous solution, strongly acidified with acetic acid, was precipitated by the addition of potassium ferrocyanide; the precipitate, however, dissolved on heating the mixture, reappearing as the solution became cool.

An aqueous solution of the albumose, acidified with acetic acid to such an extent that the first turbidity was re-dissolved, was not rendered at all turbid by the addition of a little sodium chloride; the addition of more salt, however, gave a very strong turbidity which disappeared entirely on warming, reappearing on cooling. As with the protoalbumose from fibrin, it is possible to add such a quantity of sodium chloride as to induce a very heavy precipitate, yet have it wholly disappear on boiling the mixture, separating out again, however, as the solution becomes cool. Finally the addition of a larger amount of sodium chloride gave a precipitate in the acidified solution, which was not at all affected by even boiling.

An aqueous solution of protoalbumose, when treated drop by drop with concentrated nitric acid, was rendered noticeably turbid at the point of contact, the turbidity disappearing as the mixture was shaken. On adding just the right proportion of nitric acid, a point was reached where the solution showed a permanent turbidity, which disappeared on the application of a little heat, returning as the solution cooled. A slight excess of nitric acid produced even in the cold, a very distinct reddish yellow coloration of the fluid, the turbidity disappearing. By adding crystals of salt to the acid solution, a precipitate was again formed, which disappeared on the application of heat, and reappeared as the solution cooled.

By saturating an aqueous solution of protoalbumose with salt, a heavy precipitate was formed, but in the filtrate more albumose was always found on the addition of a little acetic acid. In fact, each time protoalbumose was precipitated by sodium chloride in substance there was always a loss; a certain proportion of the substance remaining in the filtrate, precipitable only by the addition of a little acetic acid. Protoalbumose heated with acid, or treated in the cold with dilute alkalies was not apparently converted into acid albumin or alkali-albuminate-like bodies, for on neutralization, no precipitation whatever occurred. Heated with potassium hydroxide

and plumbic acetate, there was a decided blackening of the fluid. The protoalbumose likewise gave the characteristic reddish violet color with potassium hydroxide and cupric sulphate. Cupric sulphate alone, added to an aqueous solution of protoalbumose, gave a heavy greenish colored precipitate, not very soluble in excess of the copper salt. Mercuric chloride and lead acetate also precipitated the albumose.

In its reactions, therefore, the protoalbumose formed from egg-albumin does not differ, essentially at least, from fibrin protoalbumose.

### *A. Deuteroalbumose.*

This body was obtained from the first salt-saturated fluid, by the addition of a little acetic acid (sp. gr. 1042) also saturated with salt. As Kühne and Chittenden have already pointed out, all of the protoalbumose is not precipitated by saturation of a neutral fluid with sodium chloride. Hence, it is to be expected that the deuteroalbumose solution would contain some protoalbumose, which latter would be likewise precipitated by the salt-saturated acetic acid. We endeavored to make a separation, however, by rejecting altogether the first precipitate produced by the addition of a little acetic acid, and then to obtain the deuteroalbumose fairly free from the former, by the subsequent addition of more acetic acid. The final precipitate so obtained, was dissolved in a small amount of water and then dialyzed for several days. The solution, in which was noticed a small deposit of heteroalbumose, was concentrated and finally precipitated by alcohol. The precipitated deuteroalbumose was then redissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed in running water for many days, after which the solution was concentrated to a syrup, the albumose precipitated with alcohol and finally, after washing with ether, dried at 106° C. *in vacuo* until of constant weight. The composition of the product is shown in the accompanying table. The ash was composed mainly of ferric oxide and calcium phosphate; it contained no sulphate.

The pure white powder, after being dried at 106° C, was found readily soluble in water. The solution was not rendered at all turbid by saturation with sodium chloride, but the substance was more or less completely precipitated by the addition of a little acetic acid to the salt-saturated fluid. Nitric acid added to an aqueous solution of the substance gave no precipitate whatever, but colored the solution decidedly yellow even in the cold. A little sodium chloride added to

ANALYSIS OF A. DEUTEROALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5091 | 0·3168 | 6·91 | 0 9596 | 51·40 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| II | 0·4387 | 0·2725 | 6·90 | 0·8310 | 51·65 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| III | 0·5222 | ------ | ---- | ------ | ---- | 69·5 | 15·8 | 753·5 | 15·66 | ------ | ---- | ------ | ---- |
| IV | 0·6229 | ------ | ---- | ------ | ---- | 80·5 | 14·0 | 761·6 | 15·47 | ------ | ---- | ------ | ---- |
| V | 0·4525 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | 0·0566 | 1·71 | ------ | ---- |
| VI | 0.5961 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | 0·0805 | 1·85 | ------ | ---- |
| VII | 0·6743 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0078 | 1·15 |
| VIII | 0·5807 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0059 | 1·01 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·96 | 52·19 | ----- | ----- | ----- | ----- | *52·07* |
| H | 6·98 | 6·97 | ----- | ----- | ----- | ----- | *6·97* |
| N | ----- | ----- | 15·83 | 15·64 | ----- | ----- | *15·73* |
| S | ----- | ----- | ----- | ----- | 1·74 | 1·87 | *1·80* |
| O | ----- | ----- | ----- | ----- | ----- | ----- | *23·43* |
| | | | | | | | *100·00* |

the nitric acid solution gave a decided turbidity, which disappeared on warming the solution and reappeared on cooling.

The addition of acetic acid to an aqueous solution of the albumose gave no precipitate whatever, nor was any change to be observed on heating the fluid; neutralization, at least, caused no precipitation. The addition of a little sodium chloride solution to a solution of deuteroalbumose acidified slightly with acetic acid gave no precipitate whatever, but as with deuteroalbumose from fibrin, the application of a little heat induced a slight turbidity, which disappeared on raising the temperature still higher. Again, on the further addition of sodium chloride, a heavier precipitate was produced which disappeared completely on heating the solution and reappeared on cooling; and lastly, by adding more sodium chloride, a precipitate was obtained which was permanent even on heating the mixture to boiling. In these, as in nearly all other respects, the deuteroalbumose showed itself the same in nature as the deuteroalbumose from fibrin, and the reactions given for that body can well be applied here. In one reaction only was there any very noticeable difference; viz: in the reaction with cupric sulphate. Deuteroalbumose from egg-albumin gave only a slight precipitate with cupric sulphate, even on the addition of a minimum amount of the copper salt.* With acetic acid and potassium ferrocyanide, the reaction was much the same as with protoalbumose. Boiling with sodium hydroxide and lead acetate gave a decided blackening of the fluid, from the presence of sulphur.

### A. *Heteroalbumose.*

The greater portion of the heteroalbumose was obtained by the dialysis of the 5 and 10 per cent. sodium chloride solutions of the first salt precipitate, viz: in the purification of protoalbumose. Some, too, was also found in the dialysis of the precipitated deuteroalbumose. In both cases, the albumose was left as a more or less gummy precipitate, closely adherent to the parchment of the dialyser, separating out as the sodium chloride left the solution. The product was purified by solution in 5 per cent. sodium chloride, re-precipitation by the addition of salt in substance, re-solution in 5 per cent. sodium chloride and separation by dialysis, continued until all chlorine was

* This fact simply shows the greater purity of this preparation of deuteroalbumose or rather its freedom from protoalbumose, for as Dr. Neumeister has recently shown, perfectly pure deuteroalbumose gives no precipitate whatever with cupric sulphate. Later, we were able to prepare deuteroalbumose entirely free from protoalbumose.

ANALYSIS OF A. HETEROALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4441 | 0·2720 | 6·80 | 0·8324 | 51·11 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| II | 0·5365 | 0·3312 | 6·85 | 1·0047 | 51·07 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| III | 0·3226 | ----- | ---- | ------ | ---- | 42·1 | 15·6 | 754·1 | 15·39 | ------ | ---- | ------ | ---- |
| IV | 0·3255 | ----- | ---- | ------ | ---- | 41·6 | 14·5 | 757·7 | 15·20 | ------ | ---- | ------ | ---- |
| V | 0·6451 | ----- | ---- | ------ | ---- | ---- | ---- | ---- | ---- | 0·0751 | 1·59 | ------ | ---- |
| VI | 0·1772 | ----- | ---- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0033 | 1·86 |

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 52·08 | 52·08 | ---- | ---- | ---- | *52·06* |
| H | 6·93 | 6·98 | ---- | ---- | ---- | *6·95* |
| N | ---- | ---- | 15·67 | 15·48 | ---- | *15·55* |
| S | ---- | ---- | ---- | ---- | 1·68 | *1·63* |
| O | ---- | ---- | ---- | ---- | ---- | *23·81* |
| | | | | | | *100·00* |

removed from the solution. Like heteroalbumose from fibrin, this product each time it was re-dissolved in dilute sodium chloride, left a residue soluble only in dilute acids; presumably dysalbumose.

After being washed with water, alcohol and ether, the product was dried at 106° C. *in vacuo* and then analyzed with the results shown in the accompanying table.

The ash consisted mainly of calcium phosphate and a little ferric oxide, but did not contain any sulphate. The reactions of the body were found to be almost identical with those described as characteristic of heteroalbumose from fibrin.* Suspended in water or dissolved in 5–10 per cent. sodium chloride solution and then heated to boiling, the heteroalbumose was changed into a body, *coagulated heteroalbumose*, insoluble in sodium chloride but slowly soluble in 0·2 per cent. hydrochloric acid, from which it was precipitated by neutralization, apparently reconverted again, in part, into soluble heteroalbumose and in part into a body resembling dysalbumose. Thus on neutralizing the acid solution, a decided precipitate was obtained which was in part soluble in 5 per cent. sodium chloride (heteroalbumose), while the residue insoluble in the salt solution was soluble in dilute acids and in dilute alkalies (dysalbumose). In the filtrate from the neutralization precipitate, acetic acid showed the presence of still more heteroalbumose, which could be separated from the solution by dialysis.

Further, the heteroalbumose was found to be soluble in dilute acids, alkalies and alkaline carbonates, and from the solutions thus formed, it was reprecipitated by neutralization, but never completely; the amount remaining in solution being dependent naturally on the amount of neutral salt contained in the fluid.

Nitric acid precipitated the albumose, the extent of the precipitation depending on the amount of sodium chloride present. Acetic acid likewise gave a precipitate, soluble in excess of the acid. With acetic acid and potassium ferrocyanide, a precipitate was formed also soluble in excess of acid. With cupric sulphate, on the contrary, a heavy precipitate was obtained in a sodium chloride solution of the albumose, insoluble in excess of the copper salt. The substance gave the so-called biuret reaction with cupric sulphate and potassium hydroxide quite plainly, and also gave evidence of the presence of sulphur on boiling with potassium hydroxide and plumbic acetate.

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* See Zeitschrift für Biologie, Band xx, p. 32–36.

ANALYSIS OF B. PROTOALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the ash. gram. | S of Ash. % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3540 | 0·1980 | 6·21 | 0·5784 | 44·55 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| II | 0·4965 | 0·2709 | 6·06 | 0·8088 | 44·42 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| III | 0·3794 | ------ | ---- | ------ | ---- | 45·5 | 17·8 | 755·2 | 14·05 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| IV | 0·3771 | ------ | ---- | ------ | ---- | 45·1 | 18·1 | 762·8 | 14·13 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| V | 0·6098 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | 0·0773 | 12·67 | ------ | ------ | ------ | ---- | ----- |
| V*b* | 0·6098 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | 0·1018 | 2·29 | ------ | ---- | ----- |
| VI | 0·7674 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | 0·2100 | 3·75 | 1·46 |

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 51·00 | 50·88 | ---- | ---- | ---- | *50·94* |
| H | 7·11 | 6·96 | ---- | ---- | ---- | *7·03* |
| N | ---- | ---- | 16·09 | 16·18 | ---- | *16·13* |
| S | ---- | ---- | ---- | ---- | 2·01 | *2·01* |
| O | ---- | ---- | ---- | ---- | ---- | *23·89* |
| | | | | | | *100·00* |

*Digestion of albumin B.*

340 grams of the dry albumin, non-coagulated, were soaked in 2 litres of 0·4 per cent. hydrochloric acid for 24 hours, then warmed up to 45° C. and 1 litre of the purified pepsin-hydrochloric acid added, also warmed at the same temperature. The mixture was kept at 45° C. for 16 hours, then neutralized and filtered. The filtrate, containing the albumose bodies formed from the non-coagulated albumin, was then treated as already described under Albumin A.

*B. Protoalbumose.*

The protoalbumose isolated from this digestion, was purified in much the same manner as A protoalbumose, and did not differ from it in its reactions, except that with water it did not dissolve to quite so clear a solution; in fact its solution in water resembled more closely the aqueous solutions of protoalbumose from fibrin. During its final purification, it was dialyzed in running water until no chlorine reaction could be obtained with silver nitrate. In spite of this fact, however, the preparation contained a large percentage of ash, consisting mainly of calcium sulphate, ferric oxide and a little calcium phosphate.

The accompanying table shows the composition of the substance after drying at 106° C., *in vacuo*, until of constant weight.

In the purification of this protoalbumose, the substance was reprecipitated three times by saturating the aqueous solution of the precipitate with sodium chloride. By this treatment, as already stated, there is considerable loss, inasmuch as the precipitation of protoalbumose with salt in this manner is never complete, considerable remaining each time in the salt-saturated fluid. By adding a very little acetic acid, however, the protoalbumose is completely precipitated from the salt-saturated solution. The filtrates therefore, from the second and third precipitations of protoalbumose with salt alone were united, and the albumose remaining in them precipitated by the addition of a little acetic acid, saturated with sodium chloride. Our object was to see whether the protoalbumose which had at one time been precipitated by salt alone and then had finally become soluble in the salt-saturated fluid, differed at all in composition or in reaction from the protoalbumose still insoluble in the salt solution.

The albumose separated in this manner was purified by being dissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed for several days. The fluid was then con-

### Analysis of B. Protoalbumose.

Precipitated from NaCl solution, by $CH_3$–COOH.

| No. | Substance used. gram | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the ash. gram. | S of Ash. % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5802 | 0·3495 | 6·69 | 1·0523 | 49·46 | .... | .... | ..... | .... | .... | .... | ..... | ..... | ..... | .... | ..... |
| II | 0·3114 | ..... | .... | ..... | .... | 40·8 | 19·0 | 765·2 | 15·49 | .... | .... | ..... | ..... | ..... | .... | ..... |
| III | 0·4942 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0142 | 2·87 | ..... | ..... | ..... | .... | ..... |
| III*b* | 0·4942 | ..... | .... | ..... | .... | .... | .... | ..... | .... | .... | .... | 0·0091 | 0·25 | ..... | .... | ..... |
| IV | 0·5084 | ..... | .... | ..... | .... | .... | .... | ..... | .... | .... | .... | ..... | ..... | 0·0800 | 2·16 | 1·91 |

*Percentage composition of the ash-free substance.*

| | | | | Average. |
|---|---|---|---|---|
| C | 50·91 | .... | .... | *50·91* |
| H | 6·89 | .... | .... | *6·89* |
| N | .... | 15·94 | .... | *15·94* |
| S | .... | ... | 1·98 | *1·98* |
| O | .... | .... | ... | *24·28* |
| | | | | *100·00* |

centrated to a syrup and the albumose precipitated by alcohol. This precipitate was again dissolved in water, the solution made exactly neutral and again dialyzed.

After suitable concentration, the albumose was again precipitated by alcohol, washed with alcohol and ether and finally dried at 106° C. *in vacuo.*

The results of the analysis are seen in the accompanying table.

The ash in this preparation is seen to be much smaller than in the protoalbumose precipitated by salt alone. In other respects the two analyses are closely comparable, particularly the carbon and sulphur.

The reactions were in almost every case the same as with the preceding preparation, excepting perhaps a somewhat greater solubility.

### B. *Deuteroalbumose.*

This body was separated and purified in exactly the same manner as in the preceding digestion. The analysis of the product is shown in the accompanying table. The ash contained some calcium sulphate and a little ferric oxide. The reactions of the body were the same as those of A deuteroalbumose.

From this digestion, more or less heteroalbumose was separated but no analysis was made of the product, as the amount was rather small for the necessary purification.

### *Digestion of Albumin C.*

In the digestion of this sample of coagulated albumin, a much more vigorous pepsin-hydrochloric acid was employed than in the preceding digestions. The freshly coagulated albumin was placed in 3 litres of 0·4 per cent. hydrochloric acid and brought to a temperature of 45° C., then 400 c. c. of a pepsin solution, made from a pure glycerin extract of pepsin, were added and the mixture kept at 45° C. for 24 hours. The fluid was then neutralized, filtered and the clear filtrate saturated with sodium chloride. The albumose bodies were then separated and purified according to the methods already described.

The several bodies showed the same reactions as observed in the preceding preparations.

Protoalbumose and deuteroalbumose were analyzed. The results are shown in the accompanying tables. The ash of the deuteroalbumose contained no sulphate, but was composed almost entirely of ferric oxide.

ANALYSIS OF B. DEUTEROALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0.3821 | 0·2359 | 6·86 | 0·7065 | 50·42 | .... | .... | ..... | .... | .... | .... | ..... | ..... | ..... | .... | ..... |
| II | 0·2411 | 0·1480 | 6·82 | 0·4453 | 50·37 | .... | .... | ..... | .... | .... | .... | ..... | ..... | ..... | .... | ..... |
| III | 0·4945 | ..... | .... | ..... | .... | 65·2 | 18·6 | 760·1 | 15·50 | .... | .... | ..... | ..... | ..... | .... | ..... |
| IV | 0·5393 | ..... | .... | ..... | .... | 71·5 | 19·4 | 760·2 | 15.55 | .... | .... | ..... | ..... | ..... | .... | .... |
| V | 0·5424 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0085 | 1·56 | ..... | ..... | ..... | .... | ..... |
| V*b* | 0·5424 | ..... | .... | ..... | .... | .... | .... | ..... | .... | .... | .... | 0·0055 | 0·09 | ..... | .... | .... |
| VI | 0·5292 | ..... | .... | ..... | .... | .... | .... | ..... | .... | .... | .... | ..... | ..... | 0·0820 | 2·12 | 2·08 |
| VII | 0·3578 | ..... | .... | ..... | .... | .... | .... | ..... | .... | .... | .... | ..... | ..... | 0·0550 | 2·11 | 2·02 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·21 | 51·16 | .... | .... | .... | .... | *51·19* |
| H | 6·96 | 6·92 | .... | .... | .... | .... | *6·94* |
| N | .... | .... | 15·75 | 15·80 | .... | .... | *15·77* |
| S | .... | .... | .... | .... | 2·03 | 2·02 | *2·02* |
| O | .... | .... | .... | .... | .... | .... | *24·08* |
| | | | | | | | *100·00* |

*Dysalbumose.*

This form of albumose was found in all three digestions, but the amount was much smaller than noticed in the fibrin digestions. The substance was obtained as an insoluble residue, after extracting the first sodium chloride precipitate successively with 10 and 5 per cent. salt solutions and with water. It was then dissolved in 0·2 per cent. hydrochloric acid, and after filtration precipitated by neutralization. After precipitation in this manner, a portion of the substance was found soluble in sodium chloride and on dialysis of the solution, separated in much the same manner as heteroalbumose. The portion still insoluble in salt solution was then washed thoroughly with water and lastly with alcohol and ether. Not enough of the albumose was separated from any one digestion for analysis, but by uniting the products from all three, sufficient was obtained for the following analytical data:

I. 0·2537 gram substance gave 0·1500 gram $H_2O$=6·57 per cent. H and 0·4550 gram $CO_2$=48·92 per cent. C.

II. 0·3700 gram substance gave 46·3 c. c. N at 13·8° C. and 761·3$^{mm}$ pressure=14·96 per cent. N.

III. 0·1354 gram substance gave 0·0069 gram ash=5·09 per cent.

The ash-free substance therefore contained 51·52 per cent. C, 6·92 per cent. H, 15·79 per cent. N. The ash was composed wholly of ferric oxide.

The peculiar behavior of dysalbumose after solution in either dilute acids or sodium carbonate and neutralization, shows plainly that the substance is simply heteroalbumose rendered insoluble by action of the sodium chloride. Dysalbumose, wholly insoluble in sodium chloride, is readily dissolved by sodium carbonate of 1 per cent., and on neutralization of the alkaline fluid is in great part precipitated. The substance however, is now soluble in sodium chloride and has evidently been reconverted into heteroalbumose. It is very apparent, however, from our results that heteroalbumose from egg-albumin is not so readily converted into dysalbumose by the action of sodium chloride, as heteroalbumose from fibrin. In all three of our experiments, the amount found was very small.

Further, it would seem as if heteroalbumose from albumin was somewhat more resistant to the action of alcohol and ether than heteroalbumose from fibrin. Still the former did become quite rapidly insoluble in sodium chloride after standing under alcohol

ANALYSIS OF C. PROTOALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3618 | 0·2087 | 6·41 | 0·6097 | 45·95 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| II | 0·4489 | 0·2547 | 6·30 | 0·7582 | 46·05 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| III | 0·3283 | ------ | ---- | ------ | ---- | 40·9 | 17·6 | 748·1 | 14·47 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| IV | 0·3028 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | 0·0321 | 10·60 | ------ | ------ | ------ | ---- | ----- |
| IV*b* | 0·3028 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | 0·0500 | 2·26 | ------ | ---- | ----- |
| V | 0·3962 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | 0·1100 | 3·81 | 1·55 |

*Percentage composition of the ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 51·39 | 51.48 | ---- | ---- | *51·44* |
| H | 7·16 | 7·05 | ---- | ---- | *7·10* |
| N | ---- | --- | 16·18 | ---- | *16·18* |
| S | ---- | ---- | ---- | 2·00 | *2·00* |
| O | ---- | ---- | ---- | ---- | *23·28* |
| | | | | | *100·00* |

ANALYSIS OF C. DEUTEROALBUMOSE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4262 | 0·2682 | 6·99 | 0·7978 | 51·05 | .... | .... | ..... | .... | ..... | .... | ..... | .... |
| II | 0·3394 | 0·2104 | 6·88 | 0·6326 | 50·83 | ... | .... | ..... | .... | ..... | .... | ..... | .... |
| III | 0·4465 | ..... | .... | ..... | .... | 59·9 | 18·7 | 760·1 | 15·77 | ..... | .... | ..... | .... |
| IV | 0·3451 | ..... | .... | ..... | .... | 46·8 | 20·4 | 756·6 | 15·77 | ..... | .... | ..... | .... |
| V | 0·4508 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0680 | 2·07 | ..... | .... |
| VI | 0·4715 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0716 | 2·08 | ..... | .... |
| VII | 0·4769 | ..... | .... | ..... | .... | .... | .... | ..... | .... | ..... | .... | 0·0061 | 1·28 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·70 | 51·49 | .... | .... | .... | .... | *51·60* |
| H | 7·08 | 6·97 | .... | .... | .... | .... | *7·02* |
| N | .... | .... | 15·98 | 15·98 | .... | .... | *15·98* |
| S | .... | .... | .... | .... | 2·08 | 2·09 | *2·08* |
| O | .... | .... | .... | .... | .... | .... | *23·32* |
| | | | | | | | *100·00* |

for a short time. The reactions of dysalbumose, aside from its behavior towards sodium chloride, were found to be much the same as those of heteroalbumose.

### *Relation of the albumoses to albumin.*

In composition, the albumoses from albumin are seen to differ from each other somewhat more than the albumoses from fibrin; collectively, however, there is less difference in composition between the albumose bodies and the albumin from which they are formed, than noticed in the case of the albumose bodies from fibrin.* In the latter, however, there is no guarantee that the fibrin employed in the experiments had the actual composition assigned to pure blood-fibrin. The fibrin-albumoses collectively contained about 50·6 per cent. of carbon and 17·1 per cent. of nitrogen, while Hammarsten found fibrin itself to contain 52·6 per cent. of carbon and 16·9 per cent. of nitrogen. In our experiments, on the other hand, we have for comparison the composition of the albumin actually used in the experiments, and in the accompanying table the differences in composition of the various products are plainly to be seen.

Examining these in detail, we see that all of the products show a somewhat smaller content of carbon than albumin itself. With nitrogen, however, there is a very close agreement throughout, and with sulphur likewise. In the case of the fibrin-albumoses it was considered that the diminished percentage of carbon indicated plainly that the albumoses were hydration products, and that they were formed from fibrin by simple hydrolytic action. The results obtained with the globuloses did not appear to confirm this view, but in this case it must be remembered that the digestion of globulin by gastric juice may be quite a different process from albumin digestion. With albumin, however, the results, although less pronounced, also indicate hydrolytic action and that the products formed are hydration products.

The following table shows the extent of these differences, and also shows the close agreement in composition between proto- and deutero-albumose and the so-called soluble and insoluble hemialbumose from egg-albumin, isolated and analyzed by Kühne and Chittenden at the commencement of their study of these bodies.*

* See Zeitschrift für Biologie, Band xix. p. 174.

| | Protoalbumose. | | | | Deuteroalbumose. | | | Hetero-albumose. | Dys-albumose. | Albumin. |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B* | B† | C | A | B | C | A | A, B & C | Average of A, B & C |
| C | 50·98 | 50·94 | 50·91 | 51·44 | 52·07 | 51·19 | 51·60 | 52·06 | 51·52 | 52·33 |
| H | 6·90 | 7·03 | 6·89 | 7·10 | 6·97 | 6·94 | 7·02 | 6·95 | 6·92 | 6·98 |
| N | 15·77 | 16·13 | 15·94 | 16·18 | 15·78 | 15·77 | 15·98 | 15·55 | 15·79 | 15·85 |
| S | 1·81 | 2·01 | 1·98 | 2·00 | 1·80 | 2·02 | 2·08 | 1·63 | .... | 1·82 |
| O | 24·54 | 23·89 | 24·28 | 23·28 | 23·43 | 24·08 | 23·32 | 23·81 | .... | 23·02 |
| Ash | 2·50 | 12·67 | 2·87 | 10·60 | 1·08 | 1·56 | 1·28 | 1.86 | 5.09 | 0·55 |

* Sodium chloride precipitate.

† Acetic acid precipitate.

| | Fibrin products. | | | Egg-albumin products. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Proto-albumose.† | Deutero-albumose.† | Fibrin.‡ | Soluble Hemi-albumose.* | Insoluble Hemi-albumose.* | Proto-albumose.§ | Deutero-albumose.§ | Egg-albumin. |
| C | 50·77 | 50·65 | 52·68 | 50·89 | 51·04 | 51·07 | 51·62 | 52·33 |
| H | 6·78 | 6·83 | 6·83 | 6·81 | 6·89 | 6·98 | 6·97 | 6·98 |
| N | 17·14 | 17·17 | 16·91 | 15·98 | 15·79 | 16·00 | 15·82 | 15·85 |
| S | 1·08 | 0·97 | 1·10 | .... | .... | 1·95 | 1·96 | 1·82 |
| O | 24·23 | 24·38 | 22·48 | .... | .... | 24·00 | 23·63 | 23·02 |

Deuteroalbumose is seen to contain 0·7 per cent. less carbon than egg-albumin, while protoalbumose contains fully 1·25 per cent. less. The nitrogen in the two compounds is in accord with the content of carbon and the composition of the two products certainly suggests hydrolytic action.

The lower content of carbon in the albumose bodies has been explained by some writers on the ground that the precipitants used, principally acetic acid, would tend to form an acid compound, and that even when dried and ready for analysis, the compound would still contain some acid; which fact would be sufficient to account for the low content of carbon found. It is to be noticed, however, in these results that the deuteroalbumose, not only on an average but in each individual case, contains more carbon than protoalbumose, which was precipitated by sodium chloride alone without the addition of any acetic acid whatever. Further, comparing the two preparations of protoalbumose B, one of which was precipitated by salt alone and the other by acetic acid, it is seen that the percentage of carbon is the same in both. Unquestionably proto- and deuteroalbumose both do combine with acids, but after neutralization and long continued dialysis the albumose body certainly exists in a free state, uncombined with either acid or alkali.

The greater portion of this work was completed before we had any knowledge of Dr. Neumeister's work on the complete separation of protoalbumose from deuteroalbumose.‖ It was, therefore, too late for

* See Kühne and Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskörper. Zeitschrift für Biologie, Band xix, p. 174.

† Average of all the products analyzed. Zeitschrift für Biologie, Band xx, p. 40.

‡ According to Hammarsten. § Average of all products.

‖ Zur Kenntniss der Albumosen. Zeitschrift für Biologie, Band xxiii, p. 381.

us to take advantage of his method of separation. Our preparations of deuteroalbumose unquestionably were not wholly free from protoalbumose, but that they did not contain much of this body is evidenced by the small precipitate obtained with cupric sulphate; for, as Dr. Neumeister has recently shown, deuteroalbumose entirely free from protoalbumose gives no precipitate whatever with cupric sulphate.

## XXII.—Casein and its Primary Cleavage Products. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Following out the general plan of procedure indicated some time ago with other albuminous bodies,* we have endeavored to prepare and study the primary products formed in the digestion of pure casein with pepsin-hydrochloric acid. Assuming that casein in its conversion into peptone by artificial gastric juice, passes through certain intermediate stages, in which bodies akin to the albumose bodies are formed, we have applied the methods of separation used so successfully in the past and have been able to isolate a class of bodies bearing the same relationship to casein that the albumoses do to albumin. For this class of bodies we propose the name of *caseoses*.

In studying these substances and particularly their composition, we deemed it essential to be certain of the purity and composition of the casein to be digested; particularly in view of the recent controversy between Hammarsten† and A. Danilewsky as to the single nature of this albuminous body. Assuming, as claimed by Danilewsky,‡ that casein is a mixture of two albuminous bodies and that the numerous analyses recently made by Hammarsten of various preparations of casein are incorrect, particularly in the percentage of sulphur, made it incumbent on us to obtain some data on these points confirmatory of one or the other view, before advancing to a study of the products formed by the digestion of casein.

Of the various methods used for the preparation of pure casein, that depending on repeated precipitation by acids and re-solution in alkalies has been the most in vogue; for although theoretical objections might be advanced as to the possibility of change in the nature of the substance under the influence of acids and alkalies, the results obtained have in some respects, at least, been very satisfactory. Alex. Schmidt, in conjunction with Kapeller,§ showed plainly that dialysis of milk and then precipitation of the casein by acetic acid, while it gave fairly good results, was not sufficient in itself to

* See the preceding articles on globuloses and albumoses.

† See Zeitschrift für physiologische Chemie. Band vii, p. 227. Zur Frage, ob das casein ein einheitlicher stoff sei. Also same volume, p. 427.

‡ See Zeitschrift für physiologische chemie, Band vii, p. 433.

§ Beitrag zur Kenntniss der Milch, Jahresbericht für Thierchemie, 1874, p. 154

wholly remove the inorganic salts, and thus recourse was had to repeated precipitation by acids, after solution in dilute alkalies. By this method, Hammarsten* came to the conclusion that milk contains but two albuminous bodies, viz: casein and lacto-albumin, and the same investigator has repeatedly made use of this method for the preparation of pure casein. Lundberg† has plainly shown the noticeable resistance of casein to the action of acids, and Hammarsten has indicated the possibility of using acetic acid, if necessary, in place of the stronger hydrochloric acid. Millon and Commaille, however, have claimed that in the precipitation of casein with either acid, the precipitate does not consist of free casein, but is a compound of casein with the acid used.

This erroneous view, Hammarsten shows depends simply on the great difficulty of washing the precipitated casein completely, and he suggests that it is perhaps impossible to prepare large quantities of casein absolutely pure. For the preparation of the substance, however, Hammarsten recommends acetic acid in preference to hydrochloric acid and final drying of the compound at a temperature of 110° C.

Danilewsky and Radenhausen,‡ however, prefer to use hydrochloric acid in the preparation of pure casein, and for this purpose they use skimmed milk, diluted with 4–5 volumes of water, to which the dilute acid is added little by little, until a good precipitate is obtained. After filtration and repeated washing with distilled water, the casein is rubbed fine, then dissolved in water to which a little ammonia has been added, the fluid filtered and the clear filtrate again precipitated by the addition of a little dilute hydrochloric acid. Casein, so prepared, after being washed with distilled water, reacts acid to test papers and shows the usual reactions of this body; but if when freshly precipitated, the substance is boiled with perfectly neutral 50 per cent. alcohol and filtered hot, according to Danilewsky and Radenhausen, the casein is separated into two bodies, one of which is partially soluble in hot alcohol and separates out on cooling, while the other is insoluble. The soluble portion is termed caseoprotalbin, the insoluble portion caseoalbumin. The former, it is stated, gives

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* Zur Kenntniss des Caseins und der Wirkung des Labfermentes, Jahresbericht für Thierchemie, 1877, p. 158.

† Kleinere Beiträge zur Kenntniss des Caseins, Jahresbericht für Thierchemie, 1876, p. 11.

‡ Untersuchungen über die Eiweisstoffe der Milch, Jahresbericht für Thierchemie, 1880, p. 186.

no sulphur reaction when boiled with 2 per cent. sodium hydroxide, but contains 1·13 per cent. of sulphur, while caseoalbumin is stated to contain 1·23 per cent. of sulphur. Hence, according to these investigators, casein is a mixture of two bodies, one of which is rich in sulphur, while the other contains a somewhat smaller amount. The objection which these investigators make to the use of acetic acid in the preparation of casein, is that from a sodium acetate solution, casein itself is not precipitated, or only in part, but that the precipitate consists mainly of the protalbin body. Further, Danilewsky and Radenhausen claim that caseoalbumin dissolved in 1 per cent. sodium hydroxide and allowed to stand for 24 hours at the temperature of the room, is changed almost completely into caseoprotalbin, with loss of sulphur and calcium phosphate. In a similar manner, protalbin dissolved in lime water, with addition of alcohol and phosphoric acid, can be changed into caseoalbumin.

Hammarsten,* however, takes exception to these views and points out that the peculiar behavior of Danilewsky's casein towards boiling 50 per cent. alcohol, depends in part upon its content of calcium phosphate, the presence of which impurity depends upon the use of hydrochloric acid in the precipitation of the casein, which acid does not favor the removal of the salt as well as acetic acid. Using acetic acid as the precipitant and then employing a casein three times so precipitated, and which analysis showed to be almost entirely free from calcium phosphate, Hammarsten, by treatment with boiling alcohol, was unable to obtain anything more than a trace of substance corresponding to caseoalbumin. Further, casein is unquestionably changed by boiling with alcohol, as Hammarsten clearly shows; in fact it is well known that heating an albuminous body in water is liable to change its nature, at least its solubility, and there is no reason why treatment with 50 per cent. alcohol should not lead to a like result. Again, Hammarsten points out clearly another inconsistency in the reasoning of Danilewsky and Radenhausen in connection with the so-called conversion of caseoprotalbin into caseoalbumin. The former body is stated to be poorer in sulphur than the latter, and yet we are told that the protalbin body can be converted into caseoalbumin by simple solution in lime water and addition of phosphoric acid, with or without alcohol. Yet how it is possible by this method of treatment to convert a body with a small

* Zur Frage, ob das Casein ein einheitlicher Stoff sei. Zeitschrift für physiologische chemie, Band vii, p. 227.

content of sulphur into a body richer in sulphur, is hard to see. Much more plausible is it, as suggested by Hammarsten, that in these two bodies we have to deal with the same substance, in the one case united with calcium phosphate, and in the other uncombined with this salt; or in other words that the so-called protalbin body in the absence of calcium phosphate is soluble in boiling 50 per cent. alcohol, while in the presence of that salt it is insoluble.

This view being correct, and Hammarsten's observations would tend to show that it is, it is obvious that the caseoprotalbin of Danilewsky is simply a portion of the casein, which, owing to lack of a sufficient amount of calcium phosphate, passes into solution on being boiled with dilute alcohol; while caseoalbumin, on the other hand, is likewise a portion of the casein, *insoluble* on account of the presence of calcium phosphate; changed, however, more or less by action of the boiling alcohol. Further, the reason why casein precipitated several times by acetic acid does not contain as much calcium phosphate as when precipitated by hydrochloric acid, and thus reacts differently with alcohol, depends on the far greater insolubility of freshly precipitated casein in excess of acetic acid than in hydrochloric acid. In the precipitation of casein with hydrochloric acid only the slightest excess of acid can be added, on account of the ready solubility of the precipitate in this dilute acid. With acetic acid, however, a moderate excess can be added without solution of the precipitate, and thus in the latter case, a larger proportion of mineral salts are removed at each re-precipitation.

Danilewsky and Radenhausen have further called attention to the fact that casein precipitated with hydrochloric acid yields a larger amount of alkaline sulphide than when precipitated by acetic acid. This statement, Hammarsten has several times been able to verify, but the latter investigator seeks an explanation for this fact in the occasional presence of a second albuminous body, richer in sulphur, presumably serum-globulin, precipitable like casein by acids. Serum-globulin too, is readily soluble in excess of acid, even more so than casein, and hence by the acetic acid method of precipitation, which allows a far greater excess of acid, the casein would be much less liable to contamination by this hypothetical globulin than by the hydrochloric acid method. In this connection it may be well to notice that Musso and Menozzi* claim the presence in milk of a peculiar albuminous body containing 53·74 per cent. C, 15·52 per

* Studien über das Eiweiss der Milch. Jahresbericht für Thierchemie, 1878, p. 139.

cent. N and 1·55 per cent. S, for which they claim a position midway between serum-albumin and casein. It can be partially precipitated from fresh milk at ordinary temperatures by the addition of acetic acid. Further, Lebelien* has proved the presence in milk of a globulin-like body, lacto-globulin, which can be precipitated by saturating the fluid remaining after removal of the casein with sodium chloride, with magnesium sulphate. The substance appears to be identical with paraglobulin, and thus this fact, just discovered, would seem to confirm Hammarsten's theory as to the cause of the greater content of sulphur sometimes noticed in casein precipitated by hydrochloric acid.

Accepting then, Hammarsten's views as correct, it is obvious that casein precipitated by acetic acid, if not a single body, must be composed of two bodies, more or less alike and both precipitable by dilute acids. In attempting to settle this point definitely, Hammarsten has sought by analysis of a large number of preparations made under different conditions, to obtain data as to the exact composition of casein variously prepared. Naturally in this connection, considerable attention was paid to the content of sulphur, since, Danilewsky and Radenhausen's views being correct, variations in the content of sulphur would naturally be expected. If casein is a mixture of equal parts of caseoprotalbin and caseoalbumin with 1·13 per cent. and 1·23 per cent. of sulphur, respectively, then casein itself would naturally contain 1·18 per cent. of sulphur; an amount somewhat higher than has been found heretofore.

Recently, Dainlewsky† has modified his views somewhat, and now considers casein, as before, to be a mixture of two distinct bodies, but of nucleoalbumin with nucleoprotalbin instead of caseoalbumin and caseoprotalbin. As the reactions of these two bodies are apparently much the same as those given as characteristic of the caseo-bodies, this change of view appears to be mainly a change of name. Danilewsky still claims the correctness of the high content of sulphur in casein and assumes that the variation in the results obtained by different workers is due simply to difference in the methods of determination, and that unquestionably pure casein contains over 1·0 per cent. of sulphur.

The content of sulphur in casein as determined 30–40 years ago by

* Beitrag zur Kenntniss der Eiweisskörper der Kuhmilch. Jahresbericht für Thierchemie, 1885, p. 184.

† See Zeitschrift für physiologische chemie, Band vii, p. 433.

Lehmann, Rühling, Völckel and others,* varies from 0·85 per cent. to 1·10 per cent. Ritthausen,† from several analyses of the copper compound of casein, found a content of sulphur equivalent to 0·80–1·12 per cent. in the free casein. Schwarzenbach,‡ by a study of the platinum cyanide compound of casein, ascribed to casein itself a content of 0·19–1·10 per cent. of sulphur. Hammarsten, however, found by analysis of eight distinct preparations of casein, some of which had been reprecipitated even ten times with acetic acid, a content of sulphur ranging from 0·619 per cent. to 0·775 per cent.§ Later, Hammarsten‖ analyzed four other preparations of casein and each by six distinct methods. Omitting two or three results, which were altogether too low on account of inaccuracies in the method, Hammarsten found in these different preparations of casein, as a result of twenty-nine distinct determinations by five different methods, 0·798 per cent. as maximum, 0·726 per cent. as minimum, or 0·758 per cent. as the average, content of sulphur. Taking, however, the results obtained by what Hammarsten considers as the more correct methods the average content of sulphur is raised to 0·77–0·78 per cent. In no case did Hammarsten obtain results in any way confirmatory of Danilewsky's views. Hammarsten further made a large number of phosphorus determinations, and these as well as the results obtained for carbon and nitrogen showed too little variation to warrant the idea of a mixture of two bodies of unlike composition. While therefore, Hammarsten's results would seem to point conclusively to the unit-like nature of casein, we have, however, made quite a number of different preparations of the substance, both from fresh milk and from skimmed milk, with the idea of obtaining confirmatory data, with which to make direct comparisons between the composition of casein and its primary cleavage products.

### *Preparation and composition of Casein.*

The casein was precipitated in some cases by acetic and in others by hydrochloric acid. In both cases the acid used was very dilute,

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*See Gmelin-Krauts' Handbuch der Organische Chemie, Band iv, Abtheilung, iii, 1870, p. 2254.

† H. Ritthausen und R. Pott, Untersuchungen über Verbindungen der Eiweisskörper mit Kupferoxyd. Journal für prakt. Chemie, 1873, Band vii, p. 361.

‡ Annalen der Chem. u. Pharmacie, Band cxxxiii, p. 185.

§ Zeitschrift für physiologische Chemie, Band vii, p. 259.

‖ Ueber den Gehalt des caseins an Schwefel und über die Bestimmung des Schwefels in Proteinsubstanzen. Zeitschrift für physiologische chemie, Band ix, p. 273.

the hydrochloric acid being 0·2 per cent. In dissolving the casein for re-precipitation, a very dilute solution of ammonium hydroxide was employed; in fact so dilute as to consist of hardly more than water with a trace of ammonia. We used ammonia, in preference to sodium or potassium hydroxide, as this alkali would seem less liable to induce any alteration in the content of sulphur. Further, in dissolving the casein in ammonia, the solution at no time became more than very faintly, if at all alkaline; usually being hardly more than neutral to test papers.

The general method of procedure was to dilute fresh cow's milk with about four volumes of water (skimmed milk diluted considerably less) and then to precipitate the casein with either hydrochloric or acetic acids, adding the precipitant cautiously, until complete precipitation was obtained. The precipitate was then washed as completely as possible with large quantities of water, both by decantation, trituration with water in a mortar and on a cloth filter. The casein was then dissolved in the ammonia water, filtered through paper and reprecipitated, each time being thoroughly washed with water. In the portion used for analysis, the final precipitate was further washed with alcohol and ether and lastly soaked in a mixture of alcohol and ether for the more complete removal of any fat. The preparations were then dried in the air and lastly on a water bath at a gentle heat. When dry, they were powdered and extracted with boiling ether in a fat extractor for several hours, to insure complete freedom from fat. Ultimately, the products for analysis were dried at 105° C. *in vacuo* until of constant weight.

In all, seven preparations of casein were made for analysis, as follows:

No. I. From fresh milk, precipitated twice with hydrochloric acid.
" II. From fresh milk, precipitated twice with acetic acid.
" III. From skimmed milk, precipitated three times with acetic acid.
" IV. A portion of No. III, precipitated a fourth time with acetic acid.
" V. From skimmed milk, precipitated three times with hydrochloric acid.
" VI. A portion of No. V, precipitated a fourth time with hydrochloric acid.
" VII. From skimmed milk, precipitated four times with hydrochloric acid.

The methods of analysis were essentially the same as those employed by Kühne and Chittenden in the analysis of the various albumose bodies. Carbon and hydrogen were determined by combus-

tion with oxygen in an open tube, the gases passing over a long layer of granular oxide of copper at a bright red heat, a layer of lead chromate at a dull red heat and a roll of freshly reduced metallic copper. Nitrogen was determined as nitrogen gas by combustion with oxide of copper, the gases passing over a long anterior layer of heated oxide, a short layer of metallic copper and a final layer of oxide of copper. The tube was exhausted with a Sprengel pump before and after the combustion and the nitrogen was collected in a Schiffs' azotometer, provided with a jacket tube for rapid cooling of the gas to a constant temperature. In the determination of sulphur and phosphorus, the substance was fused with a mixture of potassium hydroxide and potassium nitrate (10 grams of the former and 1·5 grams of the latter) in a silver crucible, according to the method designated by Hammarsten * as 1*a*. In order to economize time, a single fusion was made to serve for both a sulphur and phosphorus determination; in other words, a sufficient amount of casein (usually 1·2 grams) was fused with potassium hydroxide and nitrate, the fused mass dissolved in water, the solution made up to a known volume and then one-half, representing one-half of the original substance, was used for the sulphur, the other half for the phosphorus determination. Both the alkali and the nitrate were free from sulphur and phosphorus; at least to such an extent that in a blank experiment, the resultant solutions gave no precipitate whatever, with either barium chloride or with molybdic solution. The oxidations were made at as low a temperature as possible, except towards the end when the temperature was raised, and occasionally a little more nitrate added, to facilitate complete oxidation.

As the percentage of sulphur was quite an important point, we took particular pains to have the final acid fluid entirely free from nitrate and nitrite, as well as from any excess of hydrochloric acid, so as to avoid as much as possible any solvent action on the barium sulphate. For the determination of sulphur, therefore, the alkaline solution of the fused mass was acidified distinctly with hydrochloric acid and the acid solution evaporated to perfect dryness on the water-bath. In this way the objectionable nitrate and nitrite were removed. The residue was then moistened with hydrochloric acid, taken up in water, and the solution allowed to stand until any chloride of silver present, had settled out. The fluid was then filtered and precipitated as usual with barium chloride. For phos-

* Zeitschrift für physiologische Chemie, Band ix, p. 289.

ANALYSIS OF CASEIN NO. I.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4104 | 0·2605 | 7·05 | 0·7935 | 52·72 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·3499 | 0·2214 | 7·03 | 0·6762 | 52·70 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·4850 | ---- | ---- | ---- | ---- | 65·6 | 20·2 | 765·2 | 15·72 | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·3751 | ---- | ---- | ---- | ---- | 50·5 | 20·0 | 763·0 | 15·80 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·6227 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0374 | 0·82 | ------ | ---- | ---- | ---- |
| VI | 0·6227 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0189 | 0·85 | ---- | ---- |
| VII | 0·4469 | ---- | ---- | ---- | ---- | ---- | --- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0043 | 0·96 |
| VIII | 0·4756 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0046 | 0·96 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·23 | 53·21 | ---- | ---- | ---- | ---- | *53·22* |
| H | 7·11 | 7·09 | ---- | ---- | ---- | ---- | *7·10* |
| N | ---- | ---- | 15·89 | 15·97 | ---- | ---- | *15·93* |
| S | ---- | ---- | ---- | ---- | 0·83 | ---- | *0·83* |
| P | ---- | ---- | ---- | ---- | ---- | 0·86 | *0·86* |
| O | ---- | ---- | --- | ---- | ---- | ---- | *22·06* |
| | | | | | | | *100·00* |

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with HOH + $KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with KOH + $KNO_3$. gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3685 | 0·2300 | 6·93 | 0·7187 | 52·81 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·4938 | 0·3120 | 7·02 | 0·9590 | 52·96 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·3653 | ---- | ---- | ---- | ---- | 49·2 | 21·2 | 758·2 | 15·66 | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·3542 | ---- | ---- | ---- | ---- | 47·5 | 20·6 | 756·5 | 15·77 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·5986 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0819 | 0·78 | ------ | ---- | ---- | ---- |
| VI | 0·7403 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0417 | 0·77 | ------ | --- | ---- | ---- |
| VII | 0·5986 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0187 | 0·87 | ---- | ---- |
| VIII | 0·7403 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0234 | 0·88 | ---- | ---- |
| IX | 0·5536 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0035 | 0·63 |
| X | 0·5324 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0030 | 0·56 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|
| C | 53·12 | 53·27 | ---- | ---- | ---- | ---- | ---- | ---- | *53·19* |
| H | 6·97 | 7·06 | --- | ---- | ---- | ---- | ---- | ---- | *7·01* |
| N | ---- | ---- | 15·75 | 15·86 | ---- | ---- | ---- | ---- | *15·80* |
| S | ---- | ---- | ---- | ---- | 0·74 | 0·78 | ---- | ---- | *0·76* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | 0·88 | 0·89 | *0·88* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *22·36* |
| | | | | | | | | | *100·00* |

ANALYSIS OF CASEIN NO. III.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | Ash found, gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3865 | 0·2451 | 7·04 | 0·7453 | 52·58 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·3805 | 0·2375 | 6·94 | 0·7335 | 52·57 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·3566 | ---- | ---- | ---- | ---- | 48·3 | 21·0 | 757·4 | 15·74 | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·3686 | ---- | ---- | ---- | ---- | 51·1 | 21.0 | 757.1 | 15·83 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·5466 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0350 | 0·88 | ------ | ---- | ---- | ---- |
| VI | 0·5466 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0172 | 0·88 | ---- | ---- |
| VII | 0·4835 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0065 | 1·34 |
| VIII | 0·4290 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0061 | 1·42 |

***Percentage composition of the ash-free substance.***

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| **C** | **53·31** | **53·30** | ---- | ---- | ---- | ---- | *53·30* |
| **H** | **7·14** | **7·04** | ---- | ---- | ---- | ---- | *7·09* |
| **N** | ---- | ---- | **15·95** | **16·04** | ---- | ---- | *15·99* |
| **S** | ---- | ---- | ---- | ---- | **0·89** | ---- | *0·89* |
| **P** | ---- | ---- | ---- | ---- | ---- | **0·89** | *0·89* |
| **O** | ---- | ---- | ---- | ---- | ---- | ---- | *21·84* |
| | | | | | | | *100·00* |

ANALYSIS OF CASEIN No. IV.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3276 | 0·2080 | 7·02 | 0·6371 | 53·03 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·4500 | 0·2807 | 6·93 | 0·8720 | 52·84 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·3642 | ---- | ---- | ---- | ---- | 48·5 | 20·4 | 764·6 | 15·66 | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·4003 | ---- | ---- | ---- | ---- | 54·1 | 20·6 | 764·6 | 15·87 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·6124 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0350 | 0·78 | ------ | ---- | ---- | ---- |
| VI | 0·6006 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0368 | 0·84 | ------ | ---- | ---- | ---- |
| VII | 0·6124 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0189 | 0·86 | ---- | ---- |
| VIII | 0·6006 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0181 | 0·87 | ---- | ---- |
| IX | 0·3887 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0084 | 0·87 |
| X | 0·3802 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0029 | 0·76 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|
| C | 53·49 | 53·30 | ---- | ---- | ---- | ---- | ---- | ---- | *53·39* |
| H | 7·11 | 7·02 | ---- | ---- | ---- | ---- | ---- | ---- | *7·06* |
| N | ---- | ---- | 15·77 | 15·98 | ---- | ---- | ---- | ---- | *15·87* |
| S | ---- | ---- | ---- | ---- | 0·79 | 0·85 | ---- | ---- | *0·82* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | 0·87 | 0·88 | *0·87* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *21·99* |
| | | | | | | | | | *100·00* |

ANALYSIS OF CASEIN No. V.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5970 | 0·3813 | 7·09 | 1·1575 | 52·87 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·6132 | ---- | ---- | 1·1934 | 52·91 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·3084 | 0·1950 | 7·02 | 0·5960 | 52·70 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·3663 | ---- | ---- | ---- | ---- | 48·8 | 18·4 | 756·9 | 15·57 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·3828 | ---- | ---- | ---- | ---- | 51·4 | 19·4 | 756·7 | 15·67 | ------ | ---- | ------ | ---- | ---- | ---- |
| VI | 0·8350 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0449 | 0·74 | ------ | ---- | ---- | ---- |
| VII | 0·8350 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0249 | 0·83 | ---- | ---- |
| VIII | 0·5279 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0045 | 0·85 |
| IX | 0·5316 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0046 | 0·86 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 53·31 | 53·35 | 53·14 | ---- | ---- | --- | ---- | *53·27* |
| H | 7·15 | ---- | 7·08 | ---- | ---- | ---- | ---- | *7·11* |
| N | ---- | ---- | ---- | 15·70 | 15·80 | ---- | ---- | *15·75* |
| S | ---- | ---- | ---- | -- | ---- | 0·75 | ---- | *0·75* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | 0·84 | *0·84* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *22·23* |
| | | | | | | | | *100·00* |

ANALYSES OF CASEIN No. VI.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3774 | 0·2857 | 6·94 | 0·7802 | 52·76 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| II | 0·3643 | 0·2294 | 6·99 | 0·7040 | 52·69 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ---- | ---- |
| III | 0·3520 | ---- | ---- | ---- | ---- | 47·8 | 19·6 | 760·3 | 15·92 | ------ | ---- | ------ | ---- | ---- | ---- |
| IV | 0·3640 | ---- | ---- | ---- | ---- | 49·7 | 20·4 | 760·3 | 15·96 | ------ | ---- | ------ | ---- | ---- | ---- |
| V | 0·6219 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0365 | 0·80 | ------ | ---- | ---- | ---- |
| VI | 0·6220 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0420 | 0·92 | ------ | ---- | ---- | ---- |
| VII | 0·6219 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0195 | 0·87 | ---- | ---- |
| VIII | 0·6220 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0193 | 0·85 | ---- | ---- |
| IX | 0·4436 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0046 | 1·03 |
| X | 0·4398 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0045 | 1·02 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|
| C | 53·29 | 53·21 | ---- | ---- | ---- | ---- | ---- | ---- | *53·25* |
| H | 7·00 | 7·05 | ---- | ---- | ---- | ---- | ---- | ---- | *7·02* |
| N | ---- | ---- | 16·07 | 16·10 | ---- | ---- | ---- | ---- | *16·08* |
| S | ---- | ---- | ---- | ---- | 0·81 | 0·93 | ---- | ---- | *0·87* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | 0·88 | 0·86 | *0·87* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *21·91* |
| | | | | | | | | | *100·00* |

ANALYSIS OF CASEIN No. VII.

| No. | Substance used. gram | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$ gram. | P % | Ash found. gram. | Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5031 | 0·3161 | 6·98 | 0·9760 | 52·90 | ---- | ---- | ------ | ---- | ------ | ---- | ------ | --- | ---- | ---- |
| II | 0·4063 | 0·2589 | 7·08 | 0·7870 | 52·82 | ---- | ---- | ------ | ---- | ------ | ---- | ------ | --- | ---- | ---- |
| III | 0·4755 | ------ | ---- | ------ | ---- | 64·1 | 20·0 | 757·3 | 15·72 | ------ | ---- | ------ | --- | ---- | ---- |
| IV | 0·3980 | ------ | ---- | ------ | ---- | 58·6 | 19·2 | 760·5 | 15·82 | ------ | ---- | ------ | --- | ---- | ---- |
| V | 0·6782 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | 0·0429 | 0·86 | ------ | --- | ---- | ---- |
| VI | 0·6782 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ------ | ---- | 0·0210 | 0·86 | ---- | ---- |
| VII | 0·4059 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ------ | --- | 0·0056 | 1·37 |
| VIII | 0·4118 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ------ | ---- | ------ | --- | 0·0052 | 1·26 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·58 | 53·49 | ----- | ---- | ----- | ----- | *53·53* |
| H | 7·07 | 7·14 | ----- | ----- | ----- | ----- | *7·10* |
| N | ----- | ----- | 15·92 | 16·01 | ----- | ----- | *15·96* |
| S | ----- | ----- | ----- | ----- | 0·88 | ----- | *0·88* |
| P | ----- | ----- | ----- | ----- | ----- | 0·88 | *0·88* |
| O | ----- | ----- | ----- | ----- | ----- | ----- | *21·65* |
| | | | | | | | *100·00* |

phorus, the alkaline fluid was acidified with nitric acid, evaporated to dryness, the residue dissolved in a little water acidified with nitric acid, filtered, and the phosphoric acid precipitated in the usual manner with ammonium molybdate. After standing 24 hours at 40° C. this precipitate was filtered off, dissolved in ammonium hydroxide and the phosphoric acid re-precipitated as ammonio magnesium phosphate and ultimately weighed as magnesium pyrophosphate. The accompanying tables show the results of the analyses of the different samples of casein.

Comparing now the average composition of these different preparations of casein, it is to be seen that they all show a very close agreement throughout. Thus the percentage of phosphorus in the seven preparations varies only from 0·84 to 0·89, sulphur from 0·75 to 0·89, nitrogen from 15·75 to 16·08, hydrogen from 7·01 to 7·11 and carbon from 53·19 to 53·53; or leaving out one preparation which for some reason showed a high content of carbon, from 53·19 to 53·39 per cent. The results therefore show a constancy in composition fully as marked as observed by Hammarsten and thus tend to confirm the latter in the view that casein is a single body of definite composition.

Comparing our results collectively, with those obtained by Hammarsten (see table showing average composition), we find a fairly close agreement throughout, although minor differences are to be observed. First, all of our preparations show a content of carbon somewhat higher than found by Hammarsten. The latter investigator found the carbon in his preparations to vary from 52·78 to 53·09 per cent., while in all of our preparations, the content of carbon calculated to the ash-free substance is above 53 per cent.. The possibility of our preparations still containing some fat was rendered improbable by the thorough treatment with ether which they had received, and further by the fact that the nitrogen in our preparations was also somewhat higher than found by Hammarsten. One of the preparations, however, with a high content of carbon, was extracted again for several hours with boiling ether, but on analysis the content of carbon was found unchanged. The content of phosphorus agrees exactly with Hammarsten's results, while the sulphur is, on an average, 0·1 per cent. higher. There is nothing in the content of sulphur, therefore, to even suggest confirmation of Danilewsky's views. The amount of ash in our preparations was somewhat larger than found by Hammarsten and further, there is no especial connection to be seen between the content of ash and the precipitant used; the

TABLE SHOWING THE AVERAGE COMPOSITION OF THE CASEINS.

| | No. I. twice precipitated with HCl. | No. II. twice precipitated with $CH_3$-COOH. | No. III. 3 times precipitated with $CH_3$-COOH. | No. IV. 4 times precipitated with $CH_3$-COOH. | No. V. 3 times precipitated with HCl. | No. VI. 4 times precipitated with HCl. | No. VII. 4 times precipitated with HCl. | Average of Nos. I–VII. | Average of Hammarsten's results.* | According to Ritthausen.† |
|---|---|---|---|---|---|---|---|---|---|---|
| C | 53·22 | 53·19 | 53·30 | 53·39 | 53·27 | 53·25 | 53·53 | 53·30 | 52·96 | 54·22 |
| H | 7·10 | 7·01 | 7·09 | 7·06 | 7·11 | 7·02 | 7·10 | 7·07 | 7·05 | 7·17 |
| N | 15·93 | 15·80 | 15·99 | 15·87 | 15·75 | 16·08 | 15·96 | 15·91 | 15·65 | 15·49 |
| S | 0·83 | 0·76 | 0·89 | 0·82 | 0·75 | 0·87 | 0·88 | 0·82 | 0·72 | 0·91 |
| P | 0·86 | 0·88 | 0·89 | 0·87 | 0·84 | 0·87 | 0·88 | 0·87 | 0·85 | ---- |
| O | 22·06 | 22·36 | 21·84 | 21·99 | 22·28 | 21·91 | 21·65 | 22·03 | 22·78 | ---- |
| ash | 0·96 | 0·59 | 1·38 | 0·81 | 0·85 | 1·02 | 1·31 | 0·98 | | |

* See Zeitschrift für physiologische chemie, Band vii, p. 269.

† Calculated by Ritthausen from analysis of the copper compound of casein, average of three results. See Jahresbericht für Thierchemie, 1873, p. 28.

amount in the acetic acid precipitate being fully as large as in the casein precipitated by hydrochloric acid.

Compared with Ritthausen's results (see table showing average composition), obtained by analysis of the copper compound of casein, the percentage of carbon comes very much too high. It is questionable, however, how close a comparison should be drawn between indirect results obtained by analysis of a metallic compound of casein and those obtained by analysis of casein itself.

In conclusion then, we must affirm that our results accord closely with those of Hammarsten's, while the two together make it very improbable that in casein we have to do with a substance composed of two bodies of unlike composition.

### *Digestion of Casein and Formation of Caseoses.*

In the digestion of casein with pepsin-hydrochloric acid, the casein was prepared by precipitation and reprecipitation with acetic acid, and rendered as pure as possible by thorough washing with water. While still moist it was placed in 0·4 per cent. hydrochloric acid, as preliminary to its treatment with pepsin. Pure pepsin solution, free from peptone and albumose, was prepared from the mucous membrane of pig's stomachs by the method already described.*

### *Digestion A.*

1300 grams of moist casein in 4 litres of 0·4 per cent. hydrochloric acid were brought to a temperature of 45° C. and 600 c.c. of pure pepsin solution added. The mixture was kept at a temperature of 45° C. throughout the digestion. The casein began almost immediately to swell up and in less than an hour the entire mixture was converted into a semi-solid, jelly-like mass. Thereupon, one litre more of 0·4 per cent. hydrochloric acid was added, together with a little more pepsin solution. At the end of three hours, the mixture was quite fluid, but contained considerable gelatinous matter in suspension. Neutralization of a filtered portion, produced no precipitate whatever. The addition of crystals of sodium chloride gave a heavy white precipitate and the filtrate from this precipitate gave a further precipitate on the addition of acetic acid. At the end of four hours, the entire mixture was made neutral with sodium hydroxide and then filtered through paper. The undigested residue, when dry, amounted to about 30 or 40 grams. This residue of so-called casein dyspeptone,

* See the preceding article on egg-albumin and albumoses.

which appeared in every digestion in greater or less quantity, was apparently wholly insoluble in fresh portions of gastric juice and was similar in its reactions to the like-body previously described by Lubavin.* When fresh, it appeared as a more or less jelly-like mass, much like starch paste. It was readily soluble in dilute alkalies and precipitated by neutralization, but insoluble in excess of acid. It was precipitated from its solution in dilute sodium hydroxide by addition of salt in substance. This body we did not attempt to study further, but hope to do so later.

The filtrate from the undigested residue, when cold, was not perfectly clear but became so on the application of a gentle heat. On boiling the solution, a very slight precipitate was formed. The addition of acids, hydrochloric, nitric or acetic, either concentrated or dilute, caused a heavy white precipitate, not wholly soluble in excess of acid, even of concentrated hydrochloric. The precipitate was likewise more or less permanent when warmed or even boiled with the acid. In the filtrate from the precipitate produced by acetic acid, the addition of potassium ferrocyanide gave no precipitate. The precipitate produced by acids was readily soluble in dilute alkalies. On addition of nitric acid, of any strength, and the application of heat even to boiling, the mixture turned first rose color then reddish, and as the boiling was continued the color deepened and finally became brownish red. The change from rose color to brown also takes place in the cold. With concentrated nitric acid the color is nearer the yellow of the xanthoprotein reaction, but still shows plainly the brown or reddish tinge.

For separation of the individual caseoses, the entire solution without being concentrated, was saturated with sodium chloride, by which an exceedingly heavy precipitate, more or less curdy, was obtained, which was finally filtered off and washed with saturated salt solution. The washing was made more thorough by grinding the mass with the salt solution in a mortar. This precipitate, by analogy, would naturally be composed mainly of a body corresponding to protoalbumose with possibly something corresponding to heteroalbumose. The precipitate was washed thoroughly with saturated salt solution, dissolved in water, filtered, and again precipitated by saturation of the fluid with sodium chloride. All of the substance, however, was not reprecipitated; quite a little remained in the salt-

* Ueber die Künstliche Pepsin-Verdauung des caseins, etc., Hoppe-Seyler's Med. Chemische Untersuchungen, p. 467.

saturated fluid and was thrown down as a white curdy precipitate by the addition of a little acetic acid, evidently some protocaseose not precipitated by salt alone. The main precipitate of protocaseose, etc., twice precipitated by salt, was treated with 3 litres of 10 per cent. salt solution, the residue with 3 litres of 5 per cent. salt solution and the residue still remaining, with 3 litres of water. In this manner, all of the proto and heterocaseose was dissolved, leaving a small residue wholly insoluble in dilute salt solutions and in water; presumably dyscaseose. The latter, however, was in exceedingly small quantity. It was dissolved in 0·2 per cent. hydrochloric acid and reprecipitated by neutralization of the solution with sodium carbonate.

### A. *Protocaseose.*

The 5 and 10 per cent. salt solutions of protocaseose together with the aqueous solution, were united and the mixture saturated with sodium chloride. Here, as before, all of the protocaseose was not precipitated; a portion remained in the filtrate and was precipitated only on the addition of a little acetic acid. The main portion of the protocaseose precipitated for the third time with salt in substance, was dissolved in water, the solution filtered and divided into two parts. One part was thymolized and dialyzed in running water until all chlorine was removed from the solution (Protocaseose A 1). The other part was again saturated with salt, the precipitate washed with saturated salt solution, then dissolved in water and like the former dialyzed until all chlorine was removed from the solution (Protocaseose A 2). In protocaseose 2, there was more evidence of the presence of a body resembling heteroalbumose than in No. 1. Thus in No. 2, quite a little gummy substance separated from the solution on dialysis, but the amount even here was not large. When the dialysis was finished, both solutions were perfectly neutral to test papers and in both cases the protocaseose was separated from the clear fluid by evaporation and precipitation with alcohol. For analysis, both products were washed thoroughly with alcohol and ether and finally dried at 105° C. *in vacuo.*

Their composition is shown in the accompanying tables.

As already stated, every time protocaseose was dissolved in water and reprecipitated by saturating the solution with sodium chloride, a certain amount of the substance remained in solution, precipitable only on addition of a little acetic acid. Protocaseose precipitated from the salt-saturated solution in this manner by acetic acid,

ANALYSIS OF PROTOCASEOSE A 1.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. | | | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | c. c. | T. °C. | Pressure mm. | | | | | |
| I | 0·4722 | 0·2851 | 6·70 | 0·8519 | 49·20 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| II | 0·3360 | ----- | ---- | 0·6080 | 49·34 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| III | 0·3500 | ----- | ---- | ------ | ---- | 43·7 | 20·4 | 766·2 | 14·71 | ------ | ---- | ------ | ---- |
| IV | 0·3850 | ----- | ---- | ------ | ---- | 48·3 | 19·7 | 766·2 | 14·81 | ------ | ---- | ------ | ---- |
| V | 0·6060 | ----- | ---- | ------ | ---- | ---- | ---- | ---- | ---- | 0·0380 | 0·86 | ------ | ---- |
| VI | 0·5025 | ----- | --- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0312 | 6·20 |

***Percentage composition of the ash-free substance.***

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 52·44 | 52·57 | ---- | ---- | ---- | *52·50* |
| H | 7·15 | ---- | ---- | ---- | ---- | *7·15* |
| N | ---- | ---- | 15·68 | 15·78 | ---- | *15·73* |
| S | ---- | ---- | ---- | ---- | 0·96 | *0·96* |
| O | ---- | ---- | ---- | ---- | ---- | *23·66* |
| | | | | | | *100·00* |

ANALYSIS OF PROTOCASEOSE A 2.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the ash. gram. | S of Ash. % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3729 | 0·2284 | 6·80 | 0·6895 | 50·42 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| II | 0·3934 | 0·2366 | 6·69 | 0·7265 | 50·38 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| III | 0·3636 | ------ | ---- | ------ | ---- | 45·8 | 20·1 | 766·1 | 14·85 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| IV | 0·3562 | ------ | ---- | ------ | ---- | 44·9 | 20·2 | 764·2 | 14·82 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| V | 0·4923 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | 0·0316 | 6·42 | ------ | ------ | ------ | ---- | ----- |
| V*b* | 0·4923 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | 0·0231 | 0·64 | ------ | ---- | ----- |
| VI | 0·5925 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | 0·0659 | 1·52 | 0·88 |

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 53·87 | 53·83 | ---- | ---- | ---- | *53·85* |
| H | 7·27 | 7·16 | ---- | ---- | ---- | *7·21* |
| N | ---- | ---- | 15·86 | 15·83 | ---- | *15·84* |
| S | ---- | ---- | ---- | ---- | 0·98 | *0·98* |
| O | ---- | ---- | ---- | ---- | ---- | *22·12* |
| | | | | | | *100·00* |

was found to be quite different in its nature from protoalbumose or protoglobulose. The two latter undoubtedly combine with acetic acid, when precipitated from a salt-saturated fluid; the compound, however, is readily and completely soluble in water. With protocaseose on the other hand, acetic acid produces a precipitate, not only insoluble in the salt-saturated fluid, but also more or less insoluble in water containing a little acid. It is easily soluble in dilute alkali and alkali carbonate, and is not precipitated by neutralization with hydrochloric or acetic acid. Addition of acid, however, beyond neutralization immediately causes precipitation of the caseose.

Protocaseose once precipitated with salt and which on the second precipitation, failed to separate from the salt-saturated fluid, was precipitated by a little acetic acid, washed somewhat with water, dissolved in very dilute sodium carbonate solution, neutralized and then dialyzed in running water for nearly a week. The solution was then perfectly neutral to test papers and was likewise perfectly clear. It was evaporated to a syrup and the caseose precipitated by alcohol. After being washed with alcohol and ether, it was dried at 105° C. *in vacuo* until of constant weight, and then analyzed with the following results:

*Protocaseose* A 3.

I. 0·3703 gram substance gave 0·2160 gram $H_2O = 6·48$ per cent. H and 0·6500 gram $CO_2 = 47·86$ per cent. C.

II. 0·4200 gram gave 50·9 c. c. N at 21·2° C. and 765·0$^{mm}$ pressure = 14·21 per cent. N.

III. 0·7053 gram fused with KOH and $KNO_3$ gave 0·0418 gram $BaSO_4$ = 0·81 per cent. S.

IV. 0·4076 gram gave 0·0386 gram ash = 9·47 per cent.

The ash-free substance therefore contained

52·59% C. 7·17% H. 15·70% N. 0·90% S.

Like all of the caseose bodies, this contained a large percentage of ash in spite of its long continued dialysis. The ash was mainly calcium phosphate with some oxide of iron, obtained in part doubtless from the salt used in precipitation. It is not difficult to see how protocaseose, precipitated by salt alone, should take up and retain semi-mechanically considerable inorganic matter. But in the present case, where the great mass of the albuminous substance has been precipitated by the salt added, it seems somewhat surprising that the caseose should separate from the clear fluid in the presence of

considerable acetic acid with such a large percentage of adherent mineral matter, unless the latter is chemically combined with the albuminous substance. Oft-repeated and long continued dialysis appears to have but little influence in diminishing the amount of this impurity. Our experience has taught us that where the caseoses have once been brought in contact with lime salts, reprecipitation and other methods of purification avail but little.

But very little heterocaseose was found in this digestion, not as much as was found in some of the others, later on. Still, during the first dialysis of the protocaseose, some little heterocaseose separated from the fluid, as the last traces of salt dialyzed out.

### A. *Deuterocaseose.*

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate of protocaseose, by acetic acid. As a study of the reactions of protocaseose had shown plainly that this body is never completely precipitated by salt alone, a little acetic acid was added to the salt-saturated filtrate, and the precipitate, presumably a mixture of proto and deuterocaseose, thrown away. The remaining deuterocaseose was then precipitated by adding about 200 c.c. of a salt-saturated acetic acid (30 per cent. acetic acid) to the fluid. The total volume of the mixture was nearly 16 litres. In this manner an abundance of a finely divided precipitate was obtained, which at first, seemed insoluble in water and in dilute sodium chloride. It was readily soluble in water containing a trace of alkali and was not precipitated by neutralization. On being washed, however, with a saturated salt solution for some time, the washings were found to have dissolved considerable of the substance, which could be precipitated from the solution by strong acetic acid. Further, after being washed with salt solution, the substance remaining appeared quite noticeably soluble in water. Evidently then, this body on being washed more or less free from acid, becomes soluble in water to a certain extent, its aqueous solution then giving a strong reaction with acetic acid and potassium ferrocyanide. The great bulk of the precipitate was therefore washed with saturated salt solution until the washings were nearly free from acid; then, having become partially soluble in water, it was placed in about 2 litres of water, the solution ultimately saturated with sodium chloride and the caseose again precipitated by addition of about 20 c. c. of acetic acid. This second precipitate was washed somewhat with salt solution and finally with a large volume of water. At first, the washings gave no reaction with

ANALYSIS OF DEUTEROCASEOSE A.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4027 | 0·2261 | 6·23 | 0·6850 | 46·38 | ---- | ---- | ----- | ---- | ----- | ---- | ----- | ----- |
| II | 0·4002 | 0·2265 | 6·27 | 0·6787 | 46·24 | ---- | ---- | ----- | ---- | ----- | ---- | ----- | ----- |
| III | 0·3736 | ----- | ---- | ----- | ---- | 45·8 | 18·0 | 765·9 | 14·07 | ----- | ---- | ----- | ----- |
| IV | 0·3550 | ----- | ---- | ----- | ---- | 42·4 | 18·4 | 766·2 | 14·17 | ----- | ---- | ----- | ----- |
| V | 0·6024 | ----- | ---- | ----- | ---- | ---- | --- | ----- | ---- | 0·0294 | 0·67 | ----- | ----- |
| VI | 0·6030 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | 0·0299 | 0·68 | ----- | ----- |
| VII | 0·3489 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | ----- | ---- | 0·0358 | 10·26 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·66 | 51·52 | ---- | ---- | ---- | ---- | *51·59* |
| H | 6·96 | 7·00 | ---- | ---- | ---- | ---- | *6·98* |
| N | ---- | ---- | 15·68 | 15·78 | ---- | ---- | *15·73* |
| S | ---- | ---- | ---- | ---- | 0·74 | 0·75 | *0·75* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *25·03* |
| | | | | | | | *100·00* |

acetic acid and potassium ferrocyanide, but later on, these reagents gave a heavy precipitate, showing plainly that the substance was dissolving. The entire precipitate was thereupon dissolved in very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed. After remaining in the dialyzers for nearly a week the fluid was removed, filtered from some heterocaseose which had separated, evaporated to a syrup on the water-bath and precipitated with alcohol. This precipitate was re-dissolved in water, the solution made exactly neutral to test papers and again dialyzed. From this solution, the caseose was finally precipitated by alcohol, after suitable concentration of the fluid, washed with alcohol and ether and dried at 105° C. *in vacuo.* The final solution, prior to precipitation by alcohol, was perfectly neutral and quite clear, showing no evidence of the presence of any heterocaseose.

The composition of the substance is shown in the accompanying table.

### *Digestion B.*

In this digestion, 750 grams of freshly prepared casein were mixed with 4 litres of 0·4 per cent. hydrochloric acid, the mixture warmed at 45° C., and then 800 c. c. of pure pepsin solution added. The mixture was warmed at the above temperature for one hour and a half, then neutralized and filtered, and the clear filtrate saturated with sodium chloride. This precipitate, as in the preceding digestion, was washed thoroughly with saturated salt solution, then successively extracted with 10 and 5 per cent. salt solution and finally with water, leaving a small residue of dyscaseose soluble only in 0·2 per cent. hydrochloric acid. The united filtrates, containing proto and heterocaseose, were again precipitated with salt and then treated as described under protocaseose B.

### *B. Protocaseose.*

The protocaseose formed in this digestion and twice precipitated by salt, was dissolved again in water, filtered through paper and then dialyzed until no chlorine reaction could be obtained with silver nitrate. Quite a little heterocaseose separated from the solution during dialysis, which was removed by filtration. The fluid was then concentrated, the substance precipitated by alcohol, again dissolved in water and dialyzed. This time, as no heterocaseose separated from the fluid, the solution was concentrated, precipitated

ANALYSIS OF PROTOCASEOSE B 1.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash % of Substance. | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3844 | 0·2268 | 6·56 | 0·6892 | 48·89 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| II | 0·4268 | 0·2504 | 6·51 | 0·7642 | 48·82 | ---- | ---- | ------ | ---- | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| III | 0·4758 | ------ | ---- | ------ | ---- | 58·5 | 19·6 | 760·8 | 14·42 | ---- | ---- | ------ | ------ | ------ | ---- | ----- |
| IV | 0·4054 | ------ | ---- | ------ | ---- | 50·3 | 20·2 | 759·8 | 14·50 | ---- | ---- | ------ | ------ | ------ | ---- | ---- |
| V | 0·3629 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | 0·0278 | 7·66 | ------ | ------ | ------ | ---- | ----- |
| V*b* | 0·3629 | ------ | ---- | ------ | ---- | ---- | ---- | ------ | ---- | ---- | ---- | 0·0200 | 0·75 | ------ | ---- | ---- |
| VI | 0·6254 | ------ | -- | ------ | ---- | ---- | --- | ------ | ---- | ---- | ---- | ------ | ------ | 0·0696 | 1·52 | 0·77 |

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 52·94 | 52·88 | ---- | ---- | ---- | *52·91* |
| H | 7·09 | 7·04 | ---- | ---- | ---- | *7·06* |
| N | ---- | ---- | 15·61 | 15·69 | ---- | *15·65* |
| S | ---- | ---- | ---- | ---- | 0·90 | *0·90* |
| O | ---- | ---- | ---- | ---- | ---- | *23·48* |
| | | | | | | *100·00* |

ANALYSIS OF PROTOCASEOSE B 2.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5084 | 0·2897 | 6·33 | 0·8808 | 47·44 | .... | .... | ..... | .... | ..... | .... | ..... | .... |
| II | 0·3520 | 0·2001 | 6·31 | 0·6130 | 47·49 | .... | .... | ..... | .... | ..... | .... | ..... | .... |
| III | 0·4521 | ..... | .... | ..... | .... | 56·0 | 19·6 | 763·5 | 14·58 | ..... | .... | ..... | .... |
| IV | 0·5010 | ..... | .... | ..... | .... | 62·5 | 20·4 | 762·3 | 14·62 | ..... | .... | ..... | .... |
| V | 0·6021 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0367 | 0·83 | ..... | .... |
| VI | 0·6010 | ..... | .... | ..... | .... | .... | .... | ..... | .... | 0·0353 | 0·81 | ..... | .... |
| VII | 0·3940 | ..... | .... | ..... | .... | .... | .... | ..... | .... | ..... | .... | 0·0389 | 9·87 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·41 | 52·46 | .... | .... | .... | .... | *52·43* |
| H | 7·02 | 7·00 | .... | .... | .... | .... | *7·01* |
| N | .... | .... | 16·17 | 16·21 | .... | .... | *16·19* |
| S | .... | .... | .... | .... | 0·91 | 0·89 | *0·90* |
| O | .... | .... | .... | .... | .... | .... | *23·47* |
| | | | | | | | *100·00* |

with alcohol and the substance finally dried at 105° C. *in vacuo* (Protocaseose B 1). Its composition is shown in the accompanying table.

Such portion of the protocaseose as was not precipitated the second time by salt alone, was precipitated by a little acetic acid. At first, the precipitated substance was insoluble in water (no reaction with acetic acid and potassium ferrocyanide), but after being washed with salt solution until the acid reaction had nearly disappeared, it then dissolved quite appreciably in water, as evidenced by the reaction with acetic acid and potassium ferrocyanide. The bulk of the precipitate, after being washed, was dissolved in a little very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed until all chlorine was removed from the solution. The caseose, after concentration of the solution, was precipitated by alcohol, washed with alcohol and ether and finally dried at 105° C. *in vacuo.* The composition of the substance (Protocaseose B 2) is shown in the accompanying table.

### *B. Deuterocaseose.*

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate, in the same manner as in the preceding digestion. Like A. deuterocaseose, this precipitate was at first insoluble in salt solution and in water, but after being washed for some time with saturated salt solution, it was found to be gradually dissolved, as shown by the rapid disappearance of the precipitate and the pronounced reaction with acetic acid and potassium ferrocyanide in the wash-fluid. Evidently the acid is easily removed from the compound by simple washing and when that has been effected, the substance becomes soluble, or else the acid compound is more insoluble in water containing a little free acid, than in water or salt solution alone. Hydrochloric acid and acetic acid seem to act alike. The substance is readily soluble in dilute sodium carbonate and is not precipitated by neutralization, but is quickly thrown down by a slight excess of hydrochloric or acetic acid. This compound was not analyzed, but was used in studying the reactions to be described later.

### *Digestion C.*

In both of the preceding digestions, the products formed resulted from the action of an exceedingly vigorous pepsin mixture. In the

present case, the pepsin solution employed was much weaker and the pepsin-casein mixture was warmed at 45° C. for several days instead of hours.

750 grams of casein were employed and 4 litres of 0·4 per cent. hydrochloric acid, to which was added a reasonable amount of pure pepsin solution. About an hour after the addition of the latter the mass began to gelatinize, and at the end of 18 hours the whole mixture was a perfectly stiff jelly. Thereupon, 2 litres more of 0·4 per cent. acid were added together with a little pepsin. The mixture was then kept at 40–45° C. for three days longer. Quite a large residue of undigested matter remained, semi-gelatinous and soluble only in alkalies. The mixture was then neutralized and filtered. The filtrate contained considerable caseose, as evidenced by the heavy precipitate obtained on saturating a portion with sodium chloride. In the preceding digestions, the caseoses were separated directly from the dilute solutions, without previous concentration, thereby avoiding any possible change due to the action of heat. In the present case, however, the perfectly neutral solution was evaporated to a small volume and then all of the caseoses were directly precipitated by saturating the fluid with ammonium sulphate. The precipitate produced was exceedingly gummy, but was washed as thoroughly as possible by trituration with a saturated solution of ammonium sulphate. The caseoses were then dissolved in water, the solution filtered from the small residue of insoluble matter and saturated with sodium chloride. The protocaseose so separated was freed from heterocaseose, etc. by repeated precipitation and dialysis.

Carbon and hydrogen were determined in a portion of the dried substance (Protocaseose C 1) with the following results:

I. 0·5107 gram substance gave 0·3101 gram $H_2O$=6·74 per cent. H and 0·9386 gram $CO_2$=50·11 per cent C.

II. 0·4088 gram gave 0·0194 gram ash = 4·80 per cent.

The ash-free substance would, therefore, contain 52·64 per cent. of carbon and 7·08 per cent. of hydrogen.

In the filtrate from the first salt precipitate, the protocaseose remaining was precipitated by the addition of a little salt-saturated acetic acid. The precipitate after being washed was dissolved in a little dilute sodium carbonate, the solution neutralized and dialyzed.

As no heterocaseose separated from the solution, it was concentrated and then precipitated by alcohol. After purification by resolution, dialysis, etc., it was dried at 105° C. *in vacuo* and analyzed with the results shown in the accompanying table (Protocaseose C 2).

ANALYSIS OF PROTOCASEOSE C 2.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4080 | 0·2658 | 6·51 | 0·7028 | 46·90 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| II | 0·4235 | 0·2458 | 6·45 | 0·7258 | 46·74 | ---- | ---- | ---- | ---- | ------ | ---- | ------ | ---- |
| III | 0·3395 | ------ | ---- | ------ | ---- | 41·4 | 19·2 | 759·4 | 14·30 | ------ | ---- | ------ | ---- |
| IV | 0·3580 | ------ | ---- | ------ | ---- | 43·9 | 20·1 | 766·2 | 14·46 | ------ | ---- | ------ | ---- |
| V | 0·6008 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | 0·0315 | 0·72 | ------ | ---- |
| VI | 0·4068 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0386 | 9·48 |
| VII | 0·4314 | ------ | ---- | ------ | ---- | ---- | ---- | ---- | ---- | ------ | ---- | 0·0399 | 9·24 |

*Percentage composition of the ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 51·81 | 51.65 | ---- | ---- | ---- | *51·73* |
| H | 7·18 | 7·12 | ---- | ---- | ---- | *7·15* |
| N | ---- | ---- | 15·77 | 15·93 | ---- | *15·85* |
| S | ---- | ---- | ---- | ---- | 0·79 | *0·79* |
| O | ---- | ---- | ---- | ---- | ---- | *24·48* |
| | | | | | | *100·00* |

Deuterocaseose was then separated from the filtrate from the acetic acid precipitate, by saturation of the fluid with ammonium sulphate, as recommended by Neumeister* for deuteroalbumose. It was then purified by dialysis, etc., and its reactions carefully studied. So far as we could see, it differed from deuterocaseose A and B in two respects only, but these points showed so marked a difference it was plainly evident that the deuterocaseose separated by ammonium sulphate was quite different from deuterocaseose A, separated by acetic acid. Thus deutero C was not precipitated at all in an aqueous solution by acetic acid, nor by acetic acid and potassium ferrocyanide, neither was its aqueous solution precipitated by cupric sulphate. The significance of these points of difference will be discussed later on. After studying the reactions, there was not enough substance remaining for analysis.

### *Digestion D.*

In this digestion, 2 kilos of freshly prepared casein were used, together with 6 litres of 0·4 per cent. hydrochloric acid and an appropriate quantity of strong pepsin solution. The mixture was warmed at 45° C. for five hours, then neutralized and filtered from the semi-gelatinous residue.

### D. *Protocaseose.*

The neutral fluid was concentrated to about 1½ litres and then filtered from the slight flocculent precipitate which had formed. Saturation of the fluid with sodium chloride gave an exceedingly heavy precipitate, somewhat more gummy than usual. The entire fluid, however, was only partially saturated with salt, with a view to see whether the precipitate produced in this manner would agree wholly with the precipitate produced on complete saturation. Thus a fractional precipitation was made, in which the first fraction represents that portion of the caseose precipitated by about two-thirds saturation of the fluid with salt. This precipitate was therefore filtered off, washed as usual, dissolved in water and re-precipitated with salt. As dyscaseose was generally found only in traces, the precipitate, after being washed, was dissolved at once in water and dialyzed until all chlorine was removed from the solution. On opening the dialyzing tubes, quite a large quantity of heterocaseose was found adherent to the sides of the paper. The clear solution of

* See Zeitschrift für Biologie, Band xxiii, p. 381.

ANALYSIS OF PROTOCASEOSE D 1.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % | $Mg_2P_2O_7$ from the ash. gram. | P of ash, % of substance. | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | P after deducting P of ash, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5211 | 0·3268 | 6·95 | 0·9890 | 51·80 | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| II | 0·4062 | 0·2508 | 6·86 | 0·7745 | 51·99 | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| III | 0·4528 | ---- | -- | ---- | ---- | 59·1 | 18·4 | 762·3 | 15·40 | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| IV | 0·5675 | ---- | -- | ---- | ---- | 48·3 | 18·5 | 762·3 | 15·50 | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| V | 0·6014 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | 0·0348 | 0·79 | ---- | -- | ---- | ---- | ----- | -- | ---- |
| VI | 0·6004 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | 0·0374 | 0·85 | ---- | -- | ---- | ---- | ----- | -- | ---- |
| VII | 0·4473 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | 0·0162 | 3·84 | ---- | ---- | ----- | -- | ---- |
| VIII | 0·4521 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | 0·0168 | 3·71 | ---- | ---- | ----- | -- | ---- |
| IX | 0·4049 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | 0·0099 | 0·68 | ----- | -- | ---- |
| X | 0·6014 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | 0·0149 | 0·69 | 0·01 |
| XI | 0·6004 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | 0·0147 | 0·68 | 0 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·84 | 54·03 | ---- | ---- | ---- | ---- | *53·93* |
| H | 7·21 | 7·12 | ---- | ---- | ---- | ---- | *7·17* |
| N | ---- | ---- | 16·00 | 16·10 | ---- | ---- | *16·05* |
| S | ---- | ---- | ---- | ---- | 0·82 | 0·88 | *0·85* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | *0* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·00* |
| | | | | | | | *100·00* |

ANALYSIS OF PROTOCASEOSE D 2.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4196 | 0·2511 | 6·65 | 0·7607 | 49.43 | ---- | --- | ----- | ----- | ----- | ---- | ----- | ---- |
| II | 0·3511 | 0·2103 | 6·65 | 0·6378 | 49.53 | ---- | --- | ----- | ----- | ----- | ---- | ----- | ---- |
| III | 0·4789 | ------ | ---- | ------ | ---- | 60·5 | 18·8 | 762·1 | 14·88 | ----- | ---- | ----- | ---- |
| IV | 0·3626 | ------ | ---- | ------ | ---- | 45·7 | 19·2 | 762·1 | 14·83 | ----- | ---- | ----- | ---- |
| V | 0·6129 | ------ | ---- | ------ | ---- | ---- | --- | ----- | ----- | 0·0414 | 0·92 | ----- | ---- |
| VI | 0·6350 | ------ | ---- | ------ | ---- | ---- | --- | ----- | ----- | 0·0482 | 1·04 | ----- | ---- |
| VII | 0·4220 | ------ | ---- | ------ | ---- | ---- | --- | ----- | ----- | ----- | ---- | 0·0267 | 6·32 |
| VIII | 0·4186 | ------ | ---- | ------ | ---- | ---- | --- | ----- | ----- | ----- | ---- | 0·0270 | 6·45 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·79 | 52·88 | ---- | ---- | ---- | ---- | *52·84* |
| H | 7·10 | 7·10 | ---- | ---- | ---- | ---- | *7·10* |
| N | ---- | ---- | 15·89 | 15·84 | ---- | ---- | *15·86* |
| S | ---- | ---- | ---- | ---- | 0·99 | 1.10 | *1·04* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *23·16* |
| | | | | | | | *100·00* |

protocaseose was concentrated, precipitated with alcohol, the precipitate dissolved in water, again precipitated with salt in substance, the precipitate dialyzed, this time without showing any heterocaseose, and finally precipitated with alcohol, washed with alcohol and ether and dried at 105° C. *in vacuo* (Protocaseose D 1). The product was analyzed with the results shown in the preceding table.

On adding more sodium chloride to the first filtrate from the above precipitate, thereby completely saturating the solution, a second precipitate of protocaseose was obtained, which was purified in the same manner as the preceding preparation. The only difference noticed while purifying the substance was that, on dialysis, nothing corresponding to heterocaseose separated from the fluid. After final washing with alcohol and ether, the substance was analyzed with the results shown in the accompanying table (Protocaseose D 2).

The difference in the results will be discussed later on.

The original salt-saturated filtrate was precipitated with a little acetic acid, and the protocaseose so precipitated dissolved in dilute sodium carbonate. The solution was then neutralized and dialyzed in running water until all chlorine was removed. The substance was then separated by precipitation with alcohol, and ultimately purified as described previously (Protocaseose D 3). The addition of a little more acetic acid to the acetic acid and salt-saturated filtrate from the above, gave a still further precipitate of caseose; presumably protocaseose with perhaps a trace of deuterocaseose, which was filtered off, washed with saturated salt solution and then freed from acid and purified in the same manner as the preceding preparation (Protocaseose D 4).

The two last products, after being dried at 105° C. *in vacuo*, were analyzed with the results shown in the following tables.

Comparing these two tables, it is seen that the difference in percentage composition of the two ash-free substances is not very great, and taking into consideration the large percentage of ash, it is probable that the two latter precipitates have approximately the same composition.

### D. *Deuterocaseose.*

The filtrate, from which certainly all protocaseose had been removed by acetic acid, and in fact nearly everything precipitable by acid from the salt-saturated fluid, was treated with ammonium sulphate in substance. A gummy precipitate resulted, which naturally enclosed considerable salt, and which for purification was dis-

ANALYSIS OF PROTOCASEOSE D 3.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure m.m. | N % | Ash found. gram. | Ash % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4072 | 0·2416 | 6·59 | 0·7199 | 48·21 | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- |
| II | 0·4384 | 0·2622 | 6·64 | 0·7767 | 48·31 | ---- | ---- | ---- | ---- | ---- | ---- | ------ | ---- |
| III | 0·3562 | ---- | ---- | ---- | ---- | 44·7 | 18·4 | 766·6 | 14·89 | ---- | ---- | ------ | ---- |
| IV | 0·3556 | ---- | ---- | ---- | ---- | 45·4 | 18·1 | 765·8 | 15·01 | ---- | ---- | ------ | ---- |
| V | 0·4036 | ---- | ---- | ---- | ---- | ---- | ---- | --- | ---- | 0·0296 | 7·33 | ------ | ---- |
| VI | 0·4614 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0337 | 1·00 |
| VII | 0·3984 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0279 | 0·96 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·01 | 52·10 | ---- | ---- | ---- | ---- | *52·05* |
| H | 7·11 | 7·16 | --- | ---- | ---- | ---- | *7·13* |
| N | ---- | ---- | 16·07 | 16·18 | ---- | ---- | *16·12* |
| S | ---- | ---- | ---- | ---- | 1·08 | 1·04 | *1·06* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *23·64* |
| | | | | | | | *100·00* |

ANALYSIS OF PROTOCASEOSE D 4.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3156 | 0·1907 | 6·71 | 0·5816 | 50·25 | ---- | ---- | ----- | ---- | ------ | ---- | ------ | ---- |
| II | 0·4575 | 0·2763 | 6·71 | 0·8408 | 50·11 | ---- | ---- | ----- | ---- | ------ | ---- | ------ | ---- |
| III | 0·3818 | ----- | ---- | ----- | ---- | 49·6 | 18·6 | 763·1 | 15·34 | ------ | ---- | ------ | ---- |
| IV | 0·3627 | ----- | ---- | ----- | ---- | 46·8 | 18·6 | 766·3 | 15·30 | ------ | ---- | ------ | ---- |
| V | 0·6093 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | 0·0418 | 0·94 | ----- | ---- |
| VI | 0·6047 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | 0·0412 | 0·93 | ----- | ---- |
| VII | 0·4237 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | ------ | ---- | 0·0217 | 5·12 |
| VIII | 0·4103 | ----- | ---- | ----- | ---- | ---- | ---- | ----- | ---- | ------ | ---- | 0·0210 | 5·11 |

***Percentage composition of the ash-free substance.***

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| **C** | **52·95** | **52·81** | ---- | ---- | ---- | ---- | ***52·88*** |
| **H** | **7·07** | **7·07** | ---- | ---- | ---- | ---- | ***7·07*** |
| **N** | ---- | ---- | **16·16** | **16·11** | ---- | ---- | ***16·13*** |
| **S** | ---- | ---- | ---- | ---- | **0·99** | **0·98** | ***0·98*** |
| **O** | ---- | ---- | ---- | ---- | ---- | ---- | ***22·94*** |
| | | | | | | | ***100·00*** |

ANALYSIS OF DEUTEROCASEOSE D.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$. gram. | S % | Ash found. gram. | Ash % | $Mg_2P_2O_7$ from the ash. gram. | P of ash, % of substance. | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$ gram. | P % | P after deducting P of ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4992 | 0·3030 | 6·74 | 0·9047 | 49·42 | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| II | 0·3644 | 0·2200 | 6·71 | 0·6591 | 49·32 | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| III | 0·4112 | ---- | -- | ---- | ---- | 52·8 | 18·5 | 766·1 | 15·23 | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| IV | 0·3860 | ---- | -- | ---- | ---- | 49·7 | 18·0 | 766·9 | 15·30 | ----- | -- | ---- | -- | ---- | ---- | ----- | -- | ---- |
| V | 0·6034 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | 0·0486 | 1·10 | ---- | -- | ---- | ---- | ----- | -- | ---- |
| VI | 0·6020 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | 0·0500 | 1·14 | ---- | -- | ---- | ---- | ----- | -- | ---- |
| VII | 0·4083 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | 0·0191 | 4·74 | ---- | ---- | ----- | -- | ---- |
| VIII | 0·4022 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | 0·0189 | 4·62 | ---- | ---- | ----- | -- | ---- |
| VIII*b* | 0·4022 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | 0·0097 | 0·67 | ----- | -- | ---- |
| IX | 0·6034 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | 0·0150 | 0·69 | 0·02 |
| X | 0·6020 | ---- | -- | ---- | ---- | -- | -- | ---- | ---- | ----- | -- | ---- | -- | ---- | ---- | 0·0151 | 0·70 | 0·03 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·84 | 51·74 | ---- | ---- | ---- | ---- | *51·79* |
| H | 7·07 | 7·04 | ---- | ---- | ---- | ---- | *7·05* |
| N | ---- | ---- | 15·97 | 16·03 | ---- | ---- | *16·00* |
| S | ---- | ---- | ---- | ---- | 1·15 | 1·19 | *1·17* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | *0* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *23·99* |
| | | | | | | | *100·00* |

solved in water and dialyzed until the greater portion of the salt was removed. The solution was then concentrated and the substance reprecipitated by saturating the neutral solution with ammonium sulphate. This precipitate, after solution in water, was then dialyzed until all of the ammonium sulphate was removed, after which the solution was concentrated and precipitated with alcohol. When dry, the substance gave by analysis the results shown in the accompanying table.

This body differs from all of the preceding preparations, in that it is not precipitated from an aqueous solution by acetic acid. Neither is it precipitated at all by the addition of salt in substance; but the addition of a little acetic acid to the salt-saturated fluid gives a heavy precipitate which, however, does not represent all of the deuterocaseose, since the filtrate gives an additional precipitate with ammonium sulphate. Apparently about one-half of the substance is precipitated by acetic acid. Further, the acetic acid precipitate in this case differs from the protocaseose precipitate with acid, in that it is readily and completely soluble in water. This body, therefore, which certainly must represent *pure* deuterocaseose, shows a close resemblance to the pure deuteroalbumose separated by Neumeister. Like the latter, it does not give any precipitate whatever with cupric sulphate nor with ferric chloride and only the faintest turbidity with acetic acid and potassium ferrocyanide.

### D. *Heterocaseose.*

In each digestion, evidence was obtained at various points in the process of separation, noticeably on dialysis of the first protoalbumose precipitate, of the presence of a body insoluble in water but soluble in dilute sodium chloride solution. The quantity of the substance, however, was in most cases exceedingly small, so much so that nothing more than a few reactions could be tried with it. In the present digestion, however, the amount was somewhat larger, and sufficed for a partial analysis. The substance was obtained as a more or less gummy residue, on dialysis of protoalbumose 1. It was purified by solution in 10 per cent. sodium chloride and separation by dialysis. Like heteroalbumose, the whole of the substance was not now soluble in salt solution, for a portion had apparently been converted into a body resembling dysalbumose, insoluble in salt solution but soluble in 0·2 per cent. hydrochloric acid.

Analysis of the dried substance gave the following results :

I. 0·4011 gram substance gave 0·2463 gram $H_2O$=6·82 per cent. H and 0·7434 gram $CO_2$=50·54 per cent. C.

II. 0·4287 gram gave 53·6 c. c. N at 18·6° C. and 760·1 mm. pressure =14·70 per cent. N.

III. 0·4118 gram gave 0·0256 gram ash =6·21 per cent.

The ash-free substance would therefore contain

53·88 per cent. C, 7·27 per cent. H, 15·67 per cent. N.

*Reactions of the caseoses.*

Under this head little need be said. The reactions characteristic of the albumose bodies in general will apply here. Certain differences, however, have already appeared in our description of the processes incident to separation of the caseoses. Protocaseose, unlike protoalbumose, is precipitated from an *aqueous* solution by acetic acid. The precipitation, however, is not complete; saturation of the acid filtrate with sodium chloride, invariably gives an additional precipitate which is the heavier of the two. Further, long-continued washing of the acid precipitate with water or salt solution appears to partially remove the acid from the caseose body. Protocaseose is likewise precipitated from an aqueous solution by hydrochloric, nitric and sulphuric acids; the precipitate, however, is far less soluble in excess of sulphuric or nitric acid than in excess of the other two acids. In very dilute acids, protocaseose is soluble and is partially precipitated by addition of stronger acid of the same kind; thus the substance is readily soluble in 0·4 per cent. hydrochloric acid, from which solution it is precipitated by the addition of a little concentrated acid, this precipitate dissolving on the addition of more acid. Evidently then, protocaseose as fast as formed by the action of pepsin-hydrochloric acid, would dissolve in the acid gastric juice and not be mixed with the jelly-like insoluble residue. Boiled with dilute or strong acid, protocaseose is apparently not changed; at least no precipitate is obtained on neutralization of the acid fluid. The acetic acid solution of protocaseose gives a very heavy precipitate with potassium ferrocyanide. In an aqueous solution of the substance, cupric sulphate gives a heavy curd-like precipitate, while ferric chloride gives a similar precipitate readily soluble in excess of the precipitant. Like protoalbumose, protocaseose is precipitated by saturation of its aqueous solution with sodium chloride, but never completely; there always remains in the

filtrate, a portion of the substance precipitable only on addition of acetic acid.

Of the several preparations of deuterocaseose, those precipitated by ammonium sulphate are evidently the only ones perfectly pure. Further, it is evident that this substance can be obtained pure only by complete removal of all protocaseose from the solution, which implies precipitation of a large portion of the deuterocaseose also, and then precipitation of the small amount of deutero remaining, by saturation of the fluid with ammonium sulphate. D deutero, prepared in this manner, shows several very marked points of difference from protocaseose. In the first place, it is not precipitated in an *aqueous* solution by acetic acid. Further, the addition of potassium ferrocyanide to a solution acidified with acetic acid gives no precipitate whatever. Cupric sulphate and ferric chloride both fail to produce any precipitate in an aqueous solution. Pure deuterocaseose, as already mentioned, is not precipitated by saturation of its aqueous solution with sodium chloride; addition of acetic acid, however, to the salt-saturated solution gives a heavy precipitate, which represents perhaps half of the deutero, the remainder of which is precipitated only by saturation of the fluid with ammonium sulphate. It is thus evident that in the precipitation of protocaseose from a salt-saturated solution by acetic acid, more or less deuterocaseose will be likewise precipitated, the amount depending probably on the concentration of the solution and other minor circumstances. Hence, A deutero is unquestionably contaminated with some protocaseose, and on the other hand protocaseose D 3 and 4, and perhaps protocaseose C 2, without doubt contain some deuterocaseose. That *A* deutero contains some protocaseose, is evident from the fact that it gives a precipitate with cupric sulphate, and further its aqueous solution is rendered decidedly turbid by acetic acid. Moreover, the protocaseose precipitated by acetic acid, and which may contain some deutero, appears to differ in one or two respects from either proto or deuterocaseose. Thus, an aqueous solution of the purified substance is precipitated like pure protocaseose by acetic acid, but the precipitate is only partially soluble in excess of the acid and even that requires a large excess.

With nitric acid, pure deuterocaseose gives no precipitate, but on warming the solution the xanthoprotein reaction comes out strongly.

### *Composition of the caseoses and their relation to casein.*

In studying the composition of the various caseoses we have been hampered by the large percentage of ash invariably present in all of

TABLE SHOWING RELATIVE COMPOSITION OF THE CASEOSES.

| *Protocaseose.* | C | H | N | S | O |
|---|---|---|---|---|---|
| A 1 NaCl precipitate | 52·50 | 7·15 | 15·73 | 0·96 | 23·66 |
| A 2 " " | 53·85 | 7·21 | 15·84 | 0·98 | 22·12 |
| A 3 Acetic acid precipitate | 52·59 | 7·17 | 15·70 | 0·90 | 23·64 |
| B 1 NaCl precipitate | 52·91 | 7·06 | 15·65 | 0·90 | 23·48 |
| B 2 Acetic acid precipitate | 52·43 | 7·01 | 16·19 | 0·90 | 23·47 |
| C 1 NaCl precipitate | 52·64 | 7·08 | .... | .... | .... |
| C 2 Acetic acid precipitate | 51·73 | 7·15 | 15·85 | 0·79 | 24·48 |
| D 1 NaCl precipitate | 53·93 | 7·17 | 16·05 | 0·85 | 22·00 |
| D 2 " " | 52·84 | 7·10 | 15·86 | 1·04 | 23·16 |
| D 3 Acetic acid precipitate | 52·05 | 7·13 | 16·12 | 1·06 | 23·64 |
| D 4 " " " | 52·88 | 7·07 | 16·13 | 0·98 | 22·94 |
| *Deuterocaseose.* | | | | | |
| A. Acetic acid precipitate | 51·59 | 6·98 | 15·73 | 0·75 | 25·03 |
| D. $(NH_4)_2SO_4$ " | 51·79 | 7·05 | 16·00 | 1·17 | 23·99 |
| *Heterocaseose.* | | | | | |
| D. | 53·88 | 7·27 | 15·67 | .... | .... |
| *Casein.* | | | | | |
| Average of Nos. I–VII | 53·30 | 7·07 | 15·91 | 0·82 | 22·03 |

the preparations. We have already commented on the difficulty, in fact, impossibility, of removing certain inorganic salts after they have once been brought in contact with a caseose body. Repeated precipitation appears to affect the percentage of ash but little. The reason for the large percentage of ash lies in the precipitation of the caseoses from such large volumes of fluid. We thought it unwise at first, to expose the bodies to the long continued evaporation necessary for precipitation with a small amount of salt. To avoid the possible danger of change, therefore, the large volumes of fluid resulting from the several digestions were saturated directly with salt, and as this involved the use of large quantities, calcium salts and some iron as impurities in the sodium chloride, were unavoidably introduced. These, the caseoses seemed at once to catch hold of and retain, in spite of oft-repeated purification. In the digestion *D*, in which the fluid was concentrated somewhat before precipitation, the percentage of ash is seen to be somewhat smaller than in preparations from the other digestions.

In comparing the composition of the individual protocaseoses (see the accompanying table) it is seen that two of the bodies show a content of carbon somewhat higher than casein itself, while the average of all the others, with one exception, shows a content of carbon a little lower than casein. Leaving out the acetic acid precipitate C 2, the average of the remaining ten preparations of protocaseose shows the following composition for this substance:

| | *C* | *H* | *N* | *S* | *O* |
|---|---|---|---|---|---|
| Protocaseose | 52·89 | 7·10 | 15·94 | 0·95 | 23·12 |
| Casein | 53·30 | 7·07 | 15·91 | 0·82 | 22·08 |

Plainly, the average of our results would indicate that protocaseose does not differ essentially in composition, from the casein from which it is formed. A slightly smaller content of carbon is the only noticeable difference. To be sure the individual results show noticeable variation in the percentage of carbon, but bearing in mind the large amount of ash present in the preparations, it is evident that the average result is of more value than the results obtained in any one case. As to the lower content of carbon in so-called protocaseose C 2, it is probable that this body is composed mainly of deuterocaseose. The two caseoses being precipitated together in this digestion by ammonium sulphate and then separated afterwards from a fairly concentrated solution by saturation with salt and addition of acetic acid, renders it probable that the protocaseose was more completely precip-

itated than usual by salt alone; and further, it is probable that on addition of acetic acid to the concentrated and salt-saturated fluid, a much larger proportion of deuterocaseose was precipitated. In confirmation of this view it was noticed that the amount of deuterocaseose obtained by the later precipitation with ammonium sulphate was quite small; far smaller proportionally than obtained in D. That the body contained some protocaseose, was evident from its reaction with cupric sulphate and with acetic acid.

Pure deuterocaseose evidently contains a smaller content of carbon than protocaseose. It is equally evident that it is a body further removed from casein than protocaseose. Its general reactions show a closer relationship to peptone than to casein or the proto-body. Heterocaseose, on the other hand, judging from analysis of a single preparation, contains fully as much if not more carbon than casein itself.

Nearly all of the caseoses show a somewhat higher percentage of sulphur than casein, but probably the increase (0·1 per cent.) is due mainly to a trace of sulphate in the ash, not accounted for. Owing to the large amount of phosphate in the ash of the different preparations, phosphorus was sought for only twice. In both of these, however (protocaseose D 1 and deuterocaseose D), the phosphorus in the ash was the exact equivalent of the total phosphorus found after fusion with potassium hydroxide and nitrate. This might indicate that in the cleavage of casein with pepsin-hydrochloric acid, the phosphorus of the casein is removed in the form of a phosphorized body, leaving the thus non-phosphorized matter to break down into the caseoses. With this thought in mind, we propose to study later the nature and composition of the insoluble, semi-gelatinous body separated in the first stage of digestion. We also hope to extend our work by a study of Weyl's commercial "casein-peptone," preliminary examination of which has shown us the presence in large quantities of caseoses. In this way and by a somewhat different method of isolating the individual caseoses, we hope to verify our present work and at the same time obtain products comparatively free from ash, with which to establish beyond question the composition of the caseoses. We are also occupied in a study of pure casein-peptone, purified according to the method made use of by Kühne and Chittenden in the study of fibrin-peptone.

## XXIII.—Influence of Some Organic and Inorganic Substances on Gas Metabolism. By R. H. Chittenden and G. W. Cummins, Ph.B.

While much time has been spent during the past few years in studying the influence of various substances on proteid metabolism, far less attention has been paid to the effects of these substances on the consumption of oxygen and the elimination of carbonic acid. Naturally in studying the influence of any substance on the nutrition of the body, we need to know not only its action on the excretion of nitrogen but also its influence on the production of carbonic acid. In this way only can we arrive at a true understanding of the influence of the substance on total metabolism, and obtain the necessary data from which to draw conclusions as to its influence on the consumption of either nitrogenous or non-nitrogenous matter. The difficulties, however, in the way of carrying on consecutive determinations of the relative amount of carbonic acid eliminated by the lungs are considerable, and in the absence of the necessary respiration apparatus, the difficulties are greatly increased. We have, however, endeavored to carry on some experiments in this direction, and although lacking the ordinary apparatus we have still been able with the means at our disposal to obtain some interesting results, a portion of which are simply confirmatory of previous work, while others are wholly new.

The apparatus employed in measuring the amount of carbonic acid eliminated is shown in the accompanying illustration (see Plate). The chamber in which the animal was placed during the experiment, was a bell jar of 32 litres capacity, with ground edge fitting closely upon a smooth glass plate. This when coated with grease made a perfectly tight joint, but in order to avoid any possibility of error, the jar and plate were placed in a shallow pan of galvanized iron, and water poured in to the depth of 2–3 inches, thus insuring a perfectly air-tight joint. In the top of the bell jar was an opening, closed with a doubly perforated rubber stopper, through which passed two tubes; one bringing air into the chamber, the other carrying it to the absorption apparatus. The inlet tube (to the left of the figure) was prolonged so as to admit the air nearly at the bottom of the jar, while the outlet tube came just through the stopper, thus insuring a perfect circulation of air. Air was drawn through the chamber by means of three aspirators,

two of which had a capacity of 15 litres and one of 7½ litres. The three aspirators working together would therefore draw through the chamber 37½ litres of air at every filling, and the flow was so regulated that 30 minutes were required to draw that amount of air through the apparatus. The flow of water from the aspirators was quite regular, since the inlet tubes went to the bottom and the air had to bubble up through the water, as the latter ran out, on the principle of Mariotte's bottle. The rate of flow was regulated by carefully changing the difference in height between the inlet tube (for air) of the aspirator and the outlet tube (for water). This of course, at the outset, was a tedious operation, but when once perfected and the apparatus permanently set up, the three aspirators ran exactly together, with a maximum variation of 15 seconds for the half-hour, which variation, however, was seldom observed. In addition, each aspirator was marked off into eight divisions, the last one of which was equal to only one-half of the others. In the two large aspirators these divisions indicated exactly the same volume, while in the small aspirator the divisions represented half the capacity of the former; but the flow of water in the latter was regulated to consume the same amount of time as in the former. Hence four minutes were required for the water to flow by each of the first seven divisions, and two minutes for the last, making a total of thirty minutes for the entire volume of water to flow from each aspirator.

The tube drawing the respired air from the chamber in which the animal was enclosed, was divided a short distance from the chamber, as seen in the figure, and two-fifths of the mixed air was drawn successively through three absorption tubes filled with a standard solution of barium hydroxide for absorption of the carbonic acid. The absorption tubes were about two-thirds of a metre long and the lower tube (*a*) contained 100 c. c. of a standard baryta solution, the middle tube (*b*) also 100 c. c. of the solution, and the upper tube (*c*) 50 c. c. The amount of carbonic acid absorbed was, at the end of the experiment, determined by titration with a standard solution of oxalic acid, using phenol-thalein as an indicator. Two titrations were made, one of the contents of tube *a* and one of the contents of the two tubes *b* and *c*. By using the three tubes, absorption of the carbonic acid was quite complete. In order to aid absorption, the air was broken into small bubbles by being forced through a small tube dipping beneath the barium hydroxide. Frequent blank experiments showed that all of the connections were perfectly tight, and further, all of the tubes being in the same position, that the flow of water

from each aspirator was perfectly uniform, and that the aspirators could be relied upon to draw the given volume of air through the apparatus in the time designated without any appreciable variation. In addition, the two-fifths drawn through the absorption tubes for determination of the carbonic acid was always exactly two-fifths of the aspirated air; since the aspirators, as already remarked, worked with perfect uniformity. Any tendency to variation, either in the time, or in the action of the individual aspirators, was noticed at the very outset of the experiment, as the water reached the level of the different marks on the aspirators, and could be at once checked or controlled by moving slightly the water outlet tube so as to either increase or diminish the difference in height between the latter and the inlet tube for air. Theoretically, variations in the temperature of the water in the aspirators might affect somewhat the volume of air analyzed, but a constant determination of the temperature of the water showed such slight variations that they did not seem to justify us in making any corrections for possible change in the amount of air aspirated. Naturally, all of the supports for the three absorption tubes were permanently placed, so that there could be no change of position; the tubes themselves placed in the same position in the holders; the volumes of baryta solution invariably the same, so as not to increase or decrease the pressure to be overcome; and lastly the aspirator tubes and stoppers fastened so as not to admit of any change. With these precautions, the results obtained, both as to the volume aspirated and the time consumed, were quite satisfactory.

As already mentioned, the total capacity of the three aspirators was 37½ litres or 5½ litres more than the capacity of the bell jar. This amount of air drawn through the chamber in 30 minutes, was more than enough to supply the largest rabbit experimented on, with the necessary amount of oxygen. But there must have been a slight accumulation of carbonic acid in the air of the chamber; this, however, was a constant factor throughout the experiments. Further, the results obtained, expressed in milligrams of $CO_2$, do not represent the total amount of carbonic acid eliminated by the rabbit during the thirty minutes of the experiment; but simply the amount of $CO_2$ contained in the 37½ litres of air aspirated during that time. Such a result, however, ought certainly to show just as plainly any influence on the elimination of carbonic acid, as a determination of absolute quantity and thus be equally valuable as an indication of influence or lack of influence on the gas metabolism of the body. Further, the results thus obtained ought to express equally as well, the comparative action of the various substances experimented with.

In every experiment, the time at which the animal was introduced into the bell jar was exactly noted and then two minutes were allowed before starting the aspirators, to make all of the connections properly. The rabbit was therefore under the bell jar, in each determination of carbonic acid, for exactly thirty-two minutes. The aspirators were started simultaneously and their progress carefully watched, in order to check any slight irregularity that might show itself.

The animals experimented with were wholly rabbits, and preliminary trials showed us plainly that it was very necessary to have them in a condition of hunger during the experiment, in order to avoid the irregularities incident to change in digestion. Further, we soon found that this was best accomplished by depriving the animal of food for three days, after which the experiment was commenced and allowed, as a rule, to extend through three consecutive days, the animal being deprived of food during the entire period. On the first of the three days, eight determinations of carbonic acid were made and the results obtained were used as a control, with which to compare the results obtained on the two following days, when the animal was being dosed with the substance experimented with. This, as a rule, we found to be the most satisfactory method of procedure, since small differences could not be relied upon as expressing anything of importance; for the varying restlessness of the confined animal, involving more or less muscular activity, would many times lead to variations in the amount of carbonic acid excreted, as may be noticed in the control experiments on those days when the animals were not dosed. Hence, the average of several consecutive results must necessarily express more correctly the average elimination of carbonic acid than any single result. Further, we deemed it better to allow the experiments to extend, as a rule, over several days and thus study the action of small, repeated doses of the various substances rather than to observe the effects of a single large dose, where violent action might naturally be expected.

The following table of results illustrates the way in which our experiments have been conducted, and at the same time shows the extent of variation, in the amount of carbonic acid, to be expected under normal circumstances from day to day. In this experiment, the rabbit had been deprived of food for three days, and the results show the amount of carbonic acid in the 37·5 litres of aspirated air for four distinct periods, during the fourth and fifth days. As already stated, the total amount of baryta solution employed in the three absorption tubes was 250 c. c., of which 100 c. c. were used in the first

or lower tube (a), while the remainder was used in the two other tubes b and c. Several solutions of oxalic acid were employed, the average strength of which was such that 1 c. c. equaled about 20 milligrams of carbonic acid.

| Time. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37.5 L. aspirated air. mg. | Body temperature. C. |
|---|---|---|---|---|---|---|---|---|
| | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| March 18. | | | | | | | | |
| 9:48 to 10:18 | 10·4 | 25·0 | 35·4 | 45·1 | 9·7 | 180·2 | 450·5 | 38·8 |
| 11:51 to 12:21 | 10·6 | 25·2 | 35·8 | 45·1 | 9·3 | 172·7 | 432·0 | 38·1 |
| 2:59 to 3:29 | 10·5 | 25·3 | 35·8 | 45·1 | 9·3 | 172·7 | 432·0 | 38·2 |
| 5:03 to 5:33 | 10·4 | 24·9 | 35·3 | 45·1 | 9·8 | 182·0 | 455·1 | 38·3 |
| March 19. | | | | | | | | |
| 8:59 to 9:29 | 10·2 | 25·1 | 35·3 | 45·1 | 9·8 | 182·0 | 455·1 | 38·3 |
| 10:53 to 11:23 | 10·9 | 25·3 | 36·2 | 45·1 | 8·9 | 165·3 | 413·4 | 37·7 |
| 2:53 to 3:23 | 10·2 | 24·2 | 34·4 | 45·1 | 10·7 | 198·7 | 496·8 | 38·7 |
| 4:51 to 5:21 | 10·6 | 24·9 | 35·5 | 45·1 | 9·6 | 178·3 | 445·9 | 38·9 |
| Average. | 10·5 | 25·0 | 35·5 | 45·1 | 9·6 | 179·0 | 447·6 | 38·4 |

*Action of uranyl nitrate.*

As stated in a preceding article,* the physiological action of uranium salts has been little studied. Experiments are now in progress to show the influence of uranium on proteid metabolism, and our present results show the influence of this substance on the excretion of carbonic acid. The rabbit first experimented with was deprived of food for three days, and on the fourth day the experiment was commenced, extending through three entire days, during which time the animal was without food. The accompanying tables show the results obtained. The body temperature was ascertained by inserting a self-registering thermometer into the rectum.

* Chittenden and Hutchinson, this volume.

A study of the first results shows plainly a decided action on the part of the uranium salt. The influence of the salt, however, manifests itself somewhat slowly, and it is not until the third day that its action becomes very pronounced, when the increased excretion of carbonic acid becomes very noticeable, accompanied with a slight rise in temperature. The first action of the uranium appears to cause a diminution in body temperature and in the amount of carbonic acid eliminated. The total amount of uranium salt given was quite large (1·175 grams in divided doses), and although no especial toxic symptoms showed themselves, the animal died on the day following the conclusion of the experiment.

A second series of experiments was tried, using smaller amounts of uranium nitrate and extending through four days, the results of which are also shown in the accompanying tables. The rabbit was deprived of food for four days prior to commencing the experiment. The amount of uranium nitrate given was considerably smaller than the quantity employed in the first series of experiments, and the animal did not suffer any permanent ill effects from its use. The following table shows the average daily result, expressed in milligrams of $CO_2$ contained in the 37·5 litres of aspirated air, together with the average body temperature.

| | | |
|---|---|---|
| May 3. | 38·9° C. | 574·3 milligrams $CO_2$ |
| " 4. | 39·0 | 540·8 " " |
| " 5. | 39·9 | 581·2 " " |
| " 6. | 38·5 | 716·3 " " |

The uranium nitrate was introduced by hypodermic injection in the following quantities:

| | | |
|---|---|---|
| May 3. | | 0 |
| " 4. | 5:18 p. m. | 0·080 gram of the salt. |
| " 5. | 8:40 a. m. | 0·090 " " |
| " 5. | 10:20 a. m. | 0·100 " " |
| " 5. | 12:40 p. m. | 0·150 " " |
| " 5. | 3:25 p. m. | 0·150 " " |
| " 5. | 5:15 p. m. | 0·200 " " |
| " 6. | | 0 |
| | | 0·770 |

In this second series of experiments it is to be noticed that the first two days are given up wholly to determining the normal excretion of carbonic acid, and the results show fully how close an agreement may be expected under normal circumstances. Taking the results

### First Series of Experiments with Uranium.

*Normal period, without uranium nitrate.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| April 19. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 9:47 to 10:17 | 6·5 | 24·8 | 31·3 | 46·3 | 15·0 | 278·9 | 697·3 | 38·7 |
| 10:45 to 11:15 | 7·8 | 25·8 | 33·6 | 46·3 | 12·7 | 235·3 | 588·3 | 38·7 |
| 11:46 to 12:16 | 7·9 | 25·8 | 33·7 | 46·3 | 12·6 | 233·4 | 583·6 | 38·6 |
| P. M. | | | | | | | | |
| 2:04 to 2:34 | 8·4 | 26·3 | 34·7 | 46·3 | 11·6 | 214·9 | 537·3 | 38·4 |
| 2:57 to 3:27 | 8·4 | 26·1 | 34·5 | 46·3 | 11·8 | 218·6 | 546·6 | 38·7 |
| 3:58 to 4:28 | 8·2 | 26·1 | 34·3 | 46·3 | 12·0 | 221·4 | 553·5 | 38·6 |
| 4:52 to 5:22 | 8·5 | 26·2 | 34·7 | 46·3 | 11·6 | 214·9 | 537·3 | 38·7 |
| Average, | 7·9 | 25·9 | 33·8 | 46·3 | 12·5 | 231·0 | 577·7 | 38·9 |
| April 20. | | *With uranium nitrate.* | | | | | | |
| A. M. | | | | | | | | |
| 9:04 to 9:34 | 7·3 | 25·9 | 33·2 | 46·3 | 13·1 | 242·7 | 606·8 | 38·4 |
| 9:57 to 10:27 | 8·3 | 26·8 | 35·1 | 46·3 | 11·2 | 207·5 | 518·8 | 38·4 |
| 10:59 to 11:29 | 9·8 | 26·6 | 36·4 | 46·3 | 9·9 | 183·4 | 458·6 | 38·2 |
| 11:53 to 12:23 | 8·7 | 26·3 | 35·0 | 46·3 | 11·3 | 209·3 | 523·5 | 38·1 |
| P. M. | | | | | | | | |
| 1:59 to 2:29 | 8·8 | 26·3 | 35·1 | 46·3 | 11·2 | 207·5 | 518·8 | 38·4 |
| 3:51 to 3:21 | 8·8 | 26·5 | 35·3 | 46·3 | 11·0 | 204·8 | 512·0 | 38·8 |
| 3:46 to 4:16 | 8·4 | 26·1 | 34·5 | 46·3 | 11·8 | 218·6 | 446·6 | 39·1 |
| 4:41 to 5:11 | 6·8 | 25·2 | 32·0 | 46·3 | 14·3 | 264·9 | 662·4 | 38·9 |
| Average, | 8·4 | 26·2 | 34·6 | 46·3 | 11·7 | 217·3 | 535·9 | 38·5 |

***With uranium nitrate—continued.***

| Date. April 21. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. | | | | | | | | |
| 9:02 to 9:32 | 5·0 | 22·4 | 27·4 | 46·3 | 18·9 | 350·1 | 875·5 | 39·2 |
| 9:58 to 10:28 | 6·0 | 24·9 | 30·9 | 46·3 | 15·4 | 285·1 | 712·8 | 38·9 |
| 10:55 to 11:25 | 6·0 | 24·7 | 30·7 | 46·3 | 15·6 | 289·0 | 722·6 | 39·0 |
| 11·47 to 12:17 | 5·7 | 24·8 | 30·5 | 46·3 | 15·8 | 292·7 | 731·9 | 39·4 |
| P. M. | | | | | | | | |
| 2:00 to 2:30 | 7·3 | 25·7 | 33·0 | 46·3 | 13·3 | 246·4 | 616·1 | 39·1 |
| 2:55 to 3:25 | 6·0 | 25·0 | 31·0 | 46·3 | 15·3 | 283·4 | 708·7 | 39·1 |
| 3:47 to 4:17 | 8·4 | 26·2 | 34·6 | 46·3 | 11·7 | 216·7 | 541·5 | 39·2 |
| 4:42 to 5:12 | 9·3 | 26·6 | 35·9 | 46·3 | 10·4 | 192·6 | 481·7 | 39·1 |
| Average, | 6·7 | 25·0 | 31·7 | 46·3 | 14·6 | 269·5 | 673·9 | 39·1 |

The following figures give the average daily result in body temperature and in the amount of carbonic acid contained in 37·5 litres of aspirated air:

| | | | |
|---|---|---|---|
| April 19, | 38·9° C. | 577·7 milligrams $CO_2$ | |
| " 20, | 38·5 " | 535·9 " | " |
| " 21, | 39·1 " | 673·9 " | " |

The uranium nitrate was introduced by hypodermic injection in the following quantities:

| | | | |
|---|---|---|---|
| April 19, | 5·40 p. m. | 0·050 gram of the salt. | |
| " 20, | 8·55 a. m. | 0·100 " | " |
| " 20, | 10·35 a. m. | 0·100 " | " |
| " 20, | 12·40 p. m. | 0·150 " | " |
| " 20, | 1·35 p. m. | 0·150 " | " |
| " 20, | 5·30 p. m. | 0·300 " | " |
| " 21, | 8·55 a. m. | 0·200 " | " |
| " 21, | 2·45 p. m. | 0·125 " | " |
| | | 1·175 | |

The animal died on the 22d.

SECOND SERIES OF EXPERIMENTS WITH URANIUM.

*Normal period, without uranium.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| May 3. | | | | | | | | |
| A. M. | | | | | | | | |
| 9:08 to 9:38 | 9·2 | 24·3 | 33·5 | 44·8 | 11·3 | 228·4 | 571·1 | 38·9 |
| 10:08 to 10:38 | 9·5 | 25·0 | 34·5 | 44·8 | 10·3 | 208·2 | 520·5 | 38·8 |
| 11:03 to 11:33 | 8·7 | 24·4 | 33·1 | 44·8 | 11·7 | 236·5 | 591·3 | 38·9 |
| 11:57 to 12:27 | 8·6 | 24·2 | 32·8 | 44·8 | 12·0 | 242·5 | 606·5 | 38·7 |
| P. M. | | | | | | | | |
| 2:07 to 2:37 | 9·7 | 25·0 | 34·7 | 44·8 | 10·1 | 204·1 | 510·4 | 38·6 |
| 2:59 to 3:29 | 8·3 | 24·2 | 32·5 | 44·8 | 12·3 | 248·6 | 621·6 | 38·9 |
| 3:50 to 4:20 | 8·7 | 24·5 | 33·2 | 44·8 | 11·6 | 234·5 | 586·3 | 38·9 |
| 4:44 to 5:14 | 8·7 | 24·5 | 33·2 | 44·8 | 11·6 | 234·5 | 586·3 | 39·4 |
| Average, | 8·9 | 24·5 | 33·4 | 44·8 | 11·4 | 229·7 | 574·3 | 38·9 |
| May 4. | *Normal period—continued.* | | | | | | | |
| A. M. | | | | | | | | |
| 8:50 to 9:20 | 9·2 | 24·7 | 33·9 | 44·8 | 10·9 | 220·3 | 550·9 | 38·9 |
| 9:49 to 10:19 | 9·5 | 24·4 | 33·9 | 44·8 | 10·9 | 220·3 | 550·9 | 38·9 |
| 10:44 to 11:14 | 8·9 | 24·6 | 33·5 | 44·8 | 11·3 | 228·4 | 571·1 | 38·9 |
| 11:37 to 12:07 | 9·5 | 24·8 | 34·3 | 44·8 | 10·5 | 212·2 | 530·6 | 38·9 |
| P. M. | | | | | | | | |
| 1:56 to 2:26 | 9·1 | 25·0 | 34·1 | 44·8 | 10·7 | 216·3 | 540·8 | 39·1 |
| 2:48 to 3:18 | 9·6 | 25·2 | 34·8 | 44·8 | 10·0 | 202·1 | 505·6 | 39·2 |
| 3:41 to 4:11 | 9·4 | 25·0 | 34·4 | 44·8 | 10·4 | 210·2 | 525·6 | 39·1 |
| 4:32 to 5:02 | 9·3 | 24·8 | 34·1 | 44·8 | 10·7 | 216·3 | 540·8 | 39·0 |
| Average, | 9·3 | 24·8 | 34·1 | 44·8 | 10·7 | 216·3 | 540·8 | 39·0 |

*With uranium nitrate.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| May 5. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 8:48 to 9:18 | 8·7 | 24·6 | 33·3 | 44·8 | 11·5 | 232·4 | 581·2 | 39·1 |
| 9:41 to 10:11 | 8·5 | 24·5 | 33·0 | 44·8 | 11·8 | 238·5 | 596·3 | 39·3 |
| 10:39 to 11:19 | 9·0 | 24·8 | 33·8 | 44·8 | 11·0 | 222·3 | 555·9 | 39·6 |
| 11:34 to 12:04 | 8·7 | 24·6 | 33·3 | 44·8 | 11·5 | 232·4 | 581·2 | 39·9 |
| P. M. | | | | | | | | |
| 1:57 to 2:27 | 8·5 | 24·7 | 33·2 | 44·8 | 11·6 | 234·4 | 586·2 | 40·0 |
| 2:49 to 3:19 | 9·0 | 24·8 | 33·8 | 44·8 | 11·0 | 222·3 | 555·9 | 40·0 |
| 3:45 to 4:15 | 8·4 | 24·6 | 33·0 | 44·8 | 11·8 | 238·5 | 596·3 | 40·4 |
| 4:40 to 5:10 | 8·6 | 24·4 | 33·0 | 44·8 | 11·8 | 238·5 | 596 3 | 40·5 |
| Average, | 8·7 | 24·6 | 33·3 | 44·8 | 11·5 | 232·4 | 581·2 | 39·9 |
| May 6. | *With uranium nitrate—continued.* | | | | | | | |
| A. M. | | | | | | | | |
| 8:52 to 9:22 | 7·0 | 22·1 | 29·1 | 44·8 | 15·7 | 317·3 | 793·4 | 40·0 |
| 9:52 to 10:22 | 5·6 | 23·3 | 28·9 | 44·8 | 15·9 | 321·4 | 803·5 | 39·6 |
| 10:48 to 11:18 | 6·9 | 24·0 | 30·9 | 44·8 | 13·9 | 280·9 | 702·5 | 38·2 |
| 11:43 to 12:13 | 6·9 | 24·0 | 30·9 | 44·8 | 13·9 | 280·9 | 702·5 | 37·9 |
| P. M. | | | | | | | | |
| 2:11 to 2:41 | 7·5 | 24·6 | 32·1 | 44·8 | 12·7 | 256·7 | 641·8 | 37·4 |
| 3:02 to 3:32 | 7·5 | 24·4 | 31·9 | 44·8 | 12·9 | 261·7 | 654·4 | 38·1 |
| Average, | 6·9 | 23·7 | 30·6 | 44·8 | 14·2 | 286·5 | 716·3 | 38·5 |

### Experiment with Cupric Sulphate.

*Normal period, without copper.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| May 10. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 9:05 to 9:35 | 11·3 | 27·7 | 39·0 | 47·7 | 8·7 | 175·8 | 439·7 | 37·9 |
| 10:00 to 10:30 | 12·3 | 28·0 | 40·3 | 47·7 | 7·4 | 149·5 | 374·0 | 37·8 |
| 10:57 to 11:27 | 11·8 | 27·8 | 39·6 | 47·7 | 8·1 | 163·7 | 409·4 | 37·8 |
| 11:49 to 12:19 | 12·4 | 28·0 | 40·4 | 47·7 | 7·3 | 147·5 | 368·9 | 37·8 |
| P. M. | | | | | | | | |
| 2:07 to 2:37 | 12·2 | 27·9 | 40·1 | 47·7 | 7·6 | 153·6 | 384·1 | 37·8 |
| 3:00 to 3:30 | 11·9 | 28·0 | 39·9 | 47·7 | 7·8 | 157·6 | 394·2 | 37·7 |
| 3:52 to 4:22 | 12·4 | 28·0 | 40·4 | 47·7 | 7·3 | 147·5 | 368·9 | 37·8 |
| 4:42 to 5:12 | 12·5 | 27·9 | 40·4 | 47·7 | 7·3 | 146·5 | 366·4 | 38·3 |
| Average, | 12·1 | 27·9 | 40·0 | 47·7 | 7·7 | 155·2 | 388·2 | 37·9 |
| May 11. | | *With cupric sulphate.* | | | | | | |
| A. M. | | | | | | | | |
| 9:04 to 9:34 | 13·0 | 28·3 | 41·3 | 47·7 | 6·4 | 129·3 | 323·4 | 37·2 |
| 10:03 to 10:33 | 12·0 | 28·0 | 40·0 | 47·7 | 7·7 | 155·6 | 389·1 | 37·3 |
| 10:58 to 11:28 | 12·7 | 28·0 | 40·7 | 47·7 | 7·0 | 141·5 | 353·8 | 38·2 |
| 11:54 to 12:24 | 12·5 | 27·8 | 40·3 | 47·7 | 7·4 | 148·5 | 371·5 | 38·3 |
| P. M. | | | | | | | | |
| 1:56 to 2:26 | 11·8 | 27·9 | 39·7 | 47·7 | 8·0 | 161·7 | 404·3 | 36·7 |
| 2:49 to 3:19 | 13·0 | 28·2 | 41·2 | 47·7 | 6·5 | 131·4 | 328·5 | 35·7 |
| 3:43 to 4:13 | 13·2 | 28·2 | 41·4 | 47·7 | 6·3 | 127·3 | 318·4 | 36·6 |
| 4:39 to 5:09 | 13·7 | 28·3 | 42·0 | 47·7 | 5·7 | 115·2 | 288·1 | 35·8 |
| Average, | 12·7 | 28·1 | 40·8 | 47·7 | 6·9 | 138·8 | 347·1 | 37·0 |

*With cupric sulphate—Continued.*

| Date. May 12. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| A. M. | | | | | | | | |
| 9:11 to 9:41 | 13·3 | 28·3 | 41·6 | 47·7 | 6·1 | 123·3 | 308·3 | 35·2 |
| 10:06 to 10:36 | 13·6 | 28·3 | 41·9 | 47·7 | 5·8 | 117·2 | 293·1 | 35·3 |
| 11:00 to 11:30 | 14·7 | 28·3 | 43·0 | 47·7 | 4·7 | 95·0 | 237·5 | 35·7 |
| 11:52 to 12:22 | 13·6 | 28·4 | 42·0 | 47·7 | 5·7 | 115·2 | 288·1 | 35·8 |
| P. M. | | | | | | | | |
| 2:15 to 2:45 | 13·7 | 28·2 | 41·9 | 47·7 | 5·8 | 117·2 | 293·1 | 35·9 |
| 3:09 to 3:39 | 13·7 | 28·3 | 42·0 | 47·7 | 5·7 | 114·2 | 285·5 | 36·1 |
| 3:59 to 4:29 | 14·4 | 28·3 | 42·7 | 47·7 | 5·0 | 101·0 | 252.7 | 36·2 |
| 4:53 to 5:23 | 14·0 | 28·4 | 42·4 | 47·7 | 5·3 | 107·1 | 267·9 | 36·2 |
| Average, | 13·9 | 28·3 | 42·2 | 47·7 | 5·5 | 111·3 | 278·3 | 35·8 |

Following are the average daily results, expressed in milligrams of $CO_2$ contained in 37·5 litres of aspirated air, together with the average body temperature:

| | | |
|---|---|---|
| May 10. | 37·9° C. | 388·2 milligrams $CO_2$ |
| " 11. | 37·0 | 347·1 " " |
| " 12. | 35·8 | 278·3 " " |

The cupric sulphate was introduced by hypodermic injection, in the following amounts:

| | | |
|---|---|---|
| May 10. | 5·34 p. m. | 0·025 gram $CuSO_4$ |
| " 11. | 8:57 a. m. | 0·005 " " |
| " 11. | 9:55 a. m. | 0·025 " " |
| " 11. | 12:29 p. m. | 0·050 " " |
| " 12. | 9:08 a. m. | 0·025 " " |
| | | 0·130 |

Rabbit died on the 13th.

of these two days for comparison, it is seen that the action of the uranium is somewhat slow, but that it produces on the first day (May 5) a noticeable rise in temperature, without any appreciable change in the elimination of carbonic acid. The full effect of the uranium, however, shows itself on the day following the last dose of the salt, and we then see the same noticeable increase in the elimination of carbonic acid noticed in the first series of experiments. We have to conclude, then, that uranium nitrate, when taken in sufficient quantity, tends to raise materially the body temperature and to increase very noticeably the excretion of carbonic acid.

### *Action of cupric sulphate.*

Falck, as quoted by Dr. H. C. Wood,* has found that cupric sulphate acts upon dogs, pigeons, rabbits and similar animals as an irritant, neurotic poison; producing great depression of temperature, with progressive general paresis ending in death, apparently from failure of respiration. Our experiments on rabbits show a marked influence of the salt in depressing body temperature and a still greater influence in diminishing the production of carbonic acid. The results of one experiment are shown in the preceding table. Although but 130 milligrams of the copper salt were used altogether, the animal finally died on the day following the conclusion of the experiment.

### *Action of arsenious oxide.*

C. Schmidt and Stürzwage† have shown by experiments on cats, that arsenious acid tends to produce a noticeable diminution in the excretion of both nitrogen and carbonic acid. Voit, however, has pointed out that in these experiments, the diminished excretion depends simply on the loss of a large portion of the food by vomiting, and Bolck‡ has shown that small doses of arsenious oxide given to hungry dogs, is wholly without influence on the decomposition of proteid matter. With large, toxic doses of arsenic, Gähtgens§ and Kossel‖ have shown that a very noticeable increase in the elimination of nitrogen takes place. These facts constitute about the sum

* Therapeutics, Materia Medica and Toxicology, p. 46.

† Moleschott's Untersuchungen, vi, p. 283.

‡ Zeitschrift für Biologie, vii, p. 430.

§ Centralblatt f. Med. Wissen., 1875, p. 529.

‖ Archiv. f. exper. Path. u. Pharm, v, p. 128.

total of our knowledge regarding the action of arsenic on tissue changes.

Our experiments were made with rabbits in a condition of hunger, deprived of food for three days prior to the experiment, and the results appear to show that arsenious acid, in the case of rabbits, has a tendency to diminish the excretion of carbonic acid, presumably through its action on the metabolic activity of the tissue cells. The amount of arsenic given was quite small and the animal seemed wholly unaffected by the poison.

*Action of potassium antimony tartrate.*

Voit* states that antimony in large doses affects proteid metabolism in the same manner as arsenic, and since Saikowsky has shown that both arsenic and antimony tend to produce a fatty degeneration of the various organs, in which presumably the fat is formed from the decomposition of proteid matter, the non-nitrogenous moiety of the albumin molecule being stored up as fat instead of being burned to carbonic acid, it seems natural to expect that these two substances when taken in large quantity at least, should like phosphorus diminish both the consumption of oxygen and the elimination of carbonic acid.

With rabbits our results with antimony certainly lead to this conclusion. Even small doses of tartar emetic quickly lead to a diminished excretion of carbonic acid and also to a noticeable fall in temperature. In the first series of experiments, the results of which are shown in the accompanying tables, the excretion of carbonic acid fell from 363·6 milligrams per 37·5 litres of aspirated air to 203·8 milligrams and with a fall in temperature of from 39° C. to 34·6° C. The total amount of tartar emetic given was 82 milligrams.

In the second series of experiments, where as before, the rabbit had been deprived of food for three days prior to the experiment, still smaller quantities of antimony were given with even more pronounced results, both in the diminution of carbonic acid and in the depression of temperature. Thus while in the normal period the excretion of carbonic acid amounted to 396 milligrams per 37·5 litres of aspirated air and with a normal temperature of 38·6° C., tartar emetic (0·055 gram) given in divided doses reduced the carbonic acid to 106·5 milligrams per 37·5 litres of aspirated air and the temperature to 27·0° C. Ackermann† has already called attention to the great decrease in animal heat produced by antimony, notably in the case of rabbits.

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* Hermann's Handbuch der Physiologie, Band vi, p. 184.

† See H. C. Wood, Therapeutics, etc., p. 158.

EXPERIMENT WITH ARSENIOUS OXIDE.

*Normal period, without arsenic.*

| Date. June 7. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 10:04 to 10:34 | 7·8 | 24·5 | 32·3 | 44·3 | 12·0 | 242·5 | 606·5 | 38·7 |
| 11:08 to 11:38 | 7·5 | 25·2 | 32·7 | 45·5 | 12·8 | 258·7 | 646·9 | 38·9 |
| 12:06 to 12:36 | 9·2 | 26·2 | 35·4 | 45·5 | 10·1 | 204·1 | 510·4 | 38·9 |
| P. M. 1:57 to 2:27 | 8·3 | 25·6 | 33·9 | 45·5 | 11·6 | 234·5 | 586·3 | 38·9 |
| 2:52 to 3:22 | 8·6 | 25·7 | 34·3 | 45·5 | 11·2 | 226·4 | 566·0 | 39·0 |
| 3:50 to 4:20 | 6·9 | 25·5 | 32·4 | 45·5 | 13.1 | 264·8 | 662·0 | 39·3 |
| 4:44 to 5:14 | 9·1 | 24·9 | 34·0 | 45·5 | 10·5 | 211·2 | 528·1 | 38·9 |
| Average, | 8·2 | 25·4 | 33·6 | 45·3 | 11·6 | 234·6 | 586·6 | 38·9 |
| June 8. | | | *With arsenious oxide.* | | | | | |
| A. M. 9:00 to 9:30 | 9·0 | 25·8 | 34·8 | 45·5 | 10·7 | 215·2 | 538·2 | 38·8 |
| 9:52 to 10:22 | 10·3 | 26·4 | 36·7 | 45·5 | 8·8 | 177·8 | 444·7 | 38·7 |
| 10:45 to 11:15 | 10·1 | 26·3 | 36·4 | 45·5 | 9·1 | 183·9 | 459·9 | 38·8 |
| 11:37 to 12:07 | 9·8 | 26·4 | 36·2 | 45·5 | 9·3 | 188·0 | 470·0 | 38·8 |
| P. M. 2:14 to 2:44 | 9·1 | 26·0 | 35·1 | 45·5 | 10·4 | 218·3 | 545·8 | 39·0 |
| 3:08 to 3:38 | 10·4 | 26·5 | 36·9 | 45·5 | 8·6 | 172·8 | 432·1 | 39·0 |
| 3:58 to 4:28 | 10·0 | 26·4 | 36·4 | 45·5 | 9·1 | 182·9 | 457·4 | 39·2 |
| 4:48 to 5:18 | 9·4 | 26·3 | 35·7 | 45·5 | 9·8 | 198·1 | 495·3 | 39·2 |
| Average, | 9·8 | 26·3 | 36·0 | 45·5 | 9·5 | 192·1 | 480·4 | 38·9 |

*With arsenious oxide—continued.*

| Date. June 9. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. | | | | | | | | |
| 9:00 to 9:30 | 9·4 | 26·1 | 35·5 | 45·5 | 10·0 | 202·1 | 505·4 | 39·0 |
| 9:52 to 10:22 | 10·4 | 26·5 | 36·9 | 45·5 | 8·6 | 172·8 | 432·1 | 38·9 |
| 10:41 to 11:11 | 9·1 | 26·1 | 35·2 | 45·5 | 10·3 | 208·2 | 520·5 | 39·1 |
| 11:36 to 12:06 | 9·6 | 26·3 | 35·9 | 45·5 | 9·6 | 194·0 | 485·2 | 39·0 |
| P. M. | | | | | | | | |
| 2:15 to 2:45 | 9·6 | 26·5 | 36·1 | 45·5 | 9·4 | 190·0 | 475·1 | 39·2 |
| 3:08 to 3:38 | 9·6 | 26·5 | 36·1 | 45·5 | 9·4 | 190·0 | 475·1 | 38·9 |
| 3:57 to 4:27 | 10·3 | 26·5 | 36·8 | 45·5 | 8·7 | 175·8 | 439·7 | 39·2 |
| 4:47 to 5:17 | 10·0 | 26·5 | 36·5 | 45·5 | 9·0 | 181·9 | 454·8 | 39·2 |
| Average, | 9·7 | 26·4 | 36·1 | 45·5 | 9·4 | 189·3 | 473·5 | 39·1 |

Average daily excretion of carbonic acid expressed in milligrams of $CO_2$ per 37·5 litres of aspirated air, and average temperature is as follows :

| | | | | |
|---|---|---|---|---|
| June 7 | 38·9° C. | 586·6 | milligrams | $CO_2$ |
| " 8 | 38·9 | 480·4 | " | " |
| " 9 | 39·1 | 473·5 | " | " |

The arsenious oxide was introduced by way of the mouth in small gelatin capsules, in the following doses :

| | | | | |
|---|---|---|---|---|
| June 7 | 5:25 p. m. | 0·005 | gram | $As_2O_3$ |
| " 8 | 8:47 a. m. | 0·005 | " | " |
| " 8 | 12:12 p. m. | 0·005 | " | " |
| " 8 | 5:25 p. m. | 0·005 | " | " |
| " 9 | 8:50 a. m. | 0·005 | " | " |
| " 9 | 12:12 p. m. | 0·010 | " | " |
| | | 0·035 | | |

FIRST SERIES OF EXPERIMENTS WITH ANTIMONY.

*Normal period, without tartar emetic.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference c. c. oxalic acid. | $CO_2$ in a, b and c mg. | $CO_2$ in 37·5 L. aspirated air. | Body temperature. |
|---|---|---|---|---|---|---|---|---|
| March 31. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 9:03 to 9:33 | 13·9 | 30·9 | 44·8 | 52·6 | 7·8 | 144·5 | 361·3 | 38·6 |
| 10:01 to 10:31 | 13·8 | 31·0 | 44·8 | 52·6 | 7·8 | 144.5 | 361·3 | 38·8 |
| 10:56 to 11:26 | 15·0 | 31·0 | 46·0 | 52·6 | 6·6 | 122·2 | 305·7 | 38·9 |
| 11:52 to 12:22 | 13·7 | 30·8 | 44·5 | 52·6 | 8·1 | 150·0 | 375·2 | 39·2 |
| P. M. | | | | | | | | |
| 1:55 to 2:25 | 12·5 | 30·0 | 42·5 | 52·6 | 10·1 | 187·0 | 467·7 | 39·1 |
| 2:47 to 3:17 | 15·0 | 31·1 | 46·1 | 52·6 | 6·5 | 120·4 | 301·1 | 39·2 |
| 3:41 to 4:11 | 14·4 | 30·4 | 44·8 | 52·6 | 7·8 | 144·5 | 361·3 | 39·2 |
| 4:36 to 5:06 | 13·7 | 30·8 | 44·5 | 52·6 | 8·1 | 150·0 | 375·2 | 39·3 |
| Average, | 14·0 | 30·7 | 44·7 | 52·6 | 7.85 | 145·4 | 363·6 | 39·0 |
| April 1. | | *With tartar emetic.* | | | | | | |
| A. M. | | | | | | | | |
| 8:57 to 9:27 | 14·1 | 30·8 | 44·9 | 52·6 | 7·7 | 141·7 | 354·4 | 38·1 |
| 9:54 to 10:24 | 14·5 | 31·0 | 45·5 | 52·6 | 7·1 | 131·5 | 328·9 | 36·9 |
| 10:50 to 11:20 | 15·4 | 31·0 | 46·4 | 52·6 | 6·2 | 114·8 | 287·2 | 36·4 |
| 11:44 to 12:24 | 15·4 | 31·0 | 46·4 | 52·6 | 6·2 | 114·8 | 287·2 | 35·7 |
| P. M. | | | | | | | | |
| 2:01 to 2:31 | 17·0 | 31·2 | 48·2 | 52·6 | 4·4 | 81·5 | 203·8 | 34·6 |
| Average, | 15·3 | 31·0 | 46·3 | 52·6 | 6·3 | 116·9 | 292·3 | 36·3 |

The antimony was given in the form of tartar emetic and was introduced by hypodermic injection as follows:

| | | | |
|---|---|---|---|
| March 31. | 5:20 p. m. | 0·012 gram tartar emetic. | |
| April 1. | 8:45 a. m. | 0·035 " " | |
| " 1. | 12:48 p. m. | 0·035 " " | |
| | | 0·082 | |

Rabbit died at 3:30 p. m., April 1.

SECOND SERIES OF EXPERIMENTS WITH ANTIMONY.

*Normal period, without tartar emetic.*

| Date. April 5. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 9:14 to 9:44 | 14·4 | 30·9 | 45·3 | 52·6 | 7·3 | 135·2 | 338·1 | 38·4 |
| 10:09 to 10:39 | 13·0 | 30·3 | 43·3 | 52·6 | 9·3 | 172·3 | 430·8 | 38·5 |
| 11:04 to 11:34 | 13·1 | 30·5 | 43·6 | 52·6 | 9·0 | 166·7 | 416·9 | 38·4 |
| 11:57 to 12:27 | 14·5 | 30·8 | 45·3 | 52·6 | 7·3 | 135·2 | 338·1 | 38·6 |
| P. M. 2:05 to 2:35 | 13·8 | 30·7 | 44·5 | 52·6 | 8·1 | 150·0 | 375·2 | 38·9 |
| 2:58 to 3:28 | 13·1 | 30·5 | 43·6 | 52·6 | 9·0 | 166·7 | 416·9 | 38·8 |
| 3:52 to 4:22 | 12·7 | 30·5 | 43·2 | 52·6 | 9·4 | 174·1 | 435·4 | 38·9 |
| 4:45 to 5:15 | 12·9 | 30·7 | 43 6 | 52·6 | 9·0 | 166·7 | 416·9 | 38·7 |
| Average, | 13·4 | 30·6 | 44·0 | 52·6 | 8·5 | 158·4 | 396·0 | 38·6 |
| April 6 | | | *With tartar emetic.* | | | | | |
| A. M. 8:59 to 9:29 | 14·5 | 30·8 | 45·3 | 52·6 | 7·3 | 135·2 | 338·1 | 37·4 |
| 9:57 to 10:27 | 15·2 | 31·1 | 46·3 | 52·6 | 6·3 | 116·7 | 291·8 | 36·7 |
| 10:52 to 11:22 | 14·5 | 30·6 | 45·1 | 52·6 | 7·5 | 138·9 | 347·4 | 36·6 |
| 11:46 to 12:16 | 14·5 | 31·0 | 45·5 | 52·6 | 7·1 | 131·5 | 328·9 | 37·3 |
| P. M. 1:58 to 2:28 | 15·3 | 30·9 | 46·2 | 52·6 | 6·35 | 117·6 | 294·1 | 36·2 |
| 2:50 to 3:20 | 14·5 | 30·9 | 45·4 | 52·6 | 7·2 | 133·4 | 333·5 | 36·2 |
| 3:42 to 4:12 | 15·4 | 31·2 | 46·6 | 52·6 | 5·95 | 110·2 | 275·6 | 35·4 |
| 4:38 to 5:08 | 15·7 | 31·2 | 46·9 | 52·6 | 5·7 | 115·6 | 289·0 | 35·7 |
| Average, | 14·9 | 31·0 | 45·9 | 52·6 | 6·68 | 124·9 | 312·2 | 36·7 |

***With tartar emetic—continued.***

| Date. April 7. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 9:00 to 9:30 | 17·6 | 31·2 | 48·8 | 52·6 | 3·8 | 70·4 | 176·0 | 30·0 |
| 9:59 to 10:29 | 18·5 | 31·3 | 49·8 | 52·6 | 2·8 | 51·8 | 129·7 | 28·0 |
| 10:53 to 11:23 | 18·9 | 31·4 | 50·3 | 52·6 | 2·3 | 42·6 | 106·5 | 27·0 |
| Average, | 18·3 | 31·3 | 49·6 | 52·6 | 2·97 | 54·9 | 137·4 | 28·3 |

Average daily excretion of carbonic acid expressed in milligrams of $CO_2$ per 37·5 litres of aspirated air, together with average temperature is as follows:

| | | | |
|---|---|---|---|
| April 5. | 38·6° C. | 396·0 | milligrams $CO_2$ |
| 6. | 36·7 | 312·0 | " " |
| 7. | 28·3 | 137·4 | " " |

The following amounts of antimony were injected:

| | | | |
|---|---|---|---|
| April 5. | 5:30 p. m. | 0·015 | gram tartar emetic. |
| 6. | 8:45 a. m. | 0·015 | " " |
| 6. | 12:39 p. m. | 0·015 | " " |
| 6. | 5:24 p. m. | 0·010 | " " |
| | | 0·055 | |

Rabbit died at 12 m., April 7.

*Action of morphine sulphate.*

Boeck and Bauer* have already made a careful study of the action of morphine on the elimination of carbonic acid and the absorption of oxygen. By experiments on a cat and on a dog they found that the action of morphine on metabolism was mainly an indirect one, affecting especially the consumption of non-nitrogenous matter. Further, that its action hinged mainly on its power of affecting muscular activity; thus in the case of a cat the first action of morphine was to increase the elimination of carbonic acid and the consumption of oxygen, due to the increased muscular activity induced by the poison, while in the case of a dog, where narcosis was half induced, there was a diminution in the amount of carbonic acid eliminated amounting in one case to 27 per cent. This diminished excretion was due almost wholly to the quieting action of the morphine and was followed by an after period in which there was increased production of carbonic acid, due to the increased activity of the muscle tissue.

In these experiments the dose of morphine was 0·05 gram, in the form of chloride, and was introduced by subcutaneous injection. The injection of the poison was followed soon after by convulsions, etc., indicating vigorous toxic action. In our first series of experiments we endeavored to have the toxic action less pronounced, and for this reason the morphine was introduced by way of the mouth in repeated doses, the experiment extending through three days and into the fourth. The rabbit was deprived of food through the entire period and had also been kept without food for three days prior to the experiment. The data are to be found in the accompanying tables.

The results do not show any very marked action, either on the excretion of carbonic acid or on the body temperature. At no time was there any noticeable indication of increased muscular activity, the rabbit remaining fairly quiet in the chamber and showing no symptoms of tetanic convulsions. On the other hand there was no very profound narcotism. A study of the individual results, however, shows that directly after each dose of morphine, the excretion of carbonic acid fell quite noticeably for one or two periods. Such action as was produced, therefore, in this experiment, is to be considered simply as incidental to the semi-somnolent condition of the animal.

In a second shorter series of experiments with a rabbit, one single

* Zeitschrift für Biologie, Band x, p. 339.

### First Series of Experiments with Morphine.

*Normal period, without morphine.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. respirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| March 24. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. 8:54 to 9:24 | 11.0 | 25·5 | 36·5 | 45·1 | 8·6 | 159·8 | 399·5 | 37·9 |
| 9:58 to 10:23 | 11·9 | 25·8 | 37·7 | 45·1 | 7·4 | 137·5 | 344·0 | 38·0 |
| 10:50 to 11:20 | 11·6 | 25·7 | 37·3 | 45·1 | 7·8 | 144·9 | 362·5 | 37·9 |
| 11:46 to 12:16 | .... | .... | 36·8 | 45·1 | 8·3 | 154·2 | 385·7 | 38·0 |
| P. M. 2:14 to 2:44 | 10·6 | 26·1 | 36·7 | 45·1 | 8·4 | 156·1 | 390·3 | 38·2 |
| 3:08 to 3:38 | 10·2 | 26·1 | 36·3 | 45·1 | 8·8 | 163·5 | 408·8 | 38·3 |
| 4:02 to 4:32 | 10·3 | 26·1 | 36·4 | 45·1 | 8·7 | 161·6 | 404·2 | 38·2 |
| 4:55 to 5:25 | 11·4 | 26·1 | 37·5 | 45·1 | 7·6 | 141·2 | 353·2 | 37·9 |
| Average, | 11·0 | 25·9 | 36·9 | 45·1 | 8·2 | 152·4 | 381·0 | 38·0 |
| March 25. | | *With morphine sulphate.* | | | | | | |
| A. M. 8:51 to 9:21 | 11·7 | 26·5 | 38·2 | 45·1 | 6·9 | 128·3 | 320·8 | 38·2 |
| 9:53 to 10:23 | 11·8 | 26·5 | 38·3 | 45·1 | 6·8 | 126·4 | 316·2 | 37·8 |
| 10:47 to 11:17 | 10·9 | 26·2 | 37·1 | 45·1 | 8·0 | 148·7 | 371·8 | 38·0 |
| 11:44 to 12:14 | 11·1 | 26·4 | 37·5 | 45·1 | 7·6 | 141 2 | 353·2 | 38·0 |
| P. M. 2:01 to 2:31 | 11·1 | 26·2 | 37·3 | 45·1 | 7.8 | 144·0 | 360·1 | 38·7 |
| 3:08 to 3:38 | 11·7 | 26·2 | 37·9 | 45·1 | 7·2 | 133·8 | 334·7 | 38·7 |
| 4:04 to 4:34 | 11·0 | 25·7 | 36·7 | 45·1 | 8·4 | 156·1 | 390·3 | 38·6 |
| 4:57 to 5:27 | 10·0 | 25·2 | 35·2 | 45·1 | 9·9 | 184·9 | 462·3 | 38·8 |
| Average, | 11·1 | 26·1 | 37·2 | 45·1 | 7·9 | 145·4 | 363·7 | 38.4 |

*With morphine sulphate—continued.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| March 26. | | | | | | | | |
| A. M. | | | | | | | | |
| 9:24 to 9:54 | 17·6 | 35·8 | 53·4 | 60·5 | 7·1 | 131·5 | 328·9 | 38·8 |
| 10:19 to 10:49 | 16·4 | 35·6 | 52·0 | 60·5 | 8·5 | 157·4 | 393·7 | 38·8 |
| 11:14 to 11:44 | 16·5 | 35·7 | 52·2 | 60·5 | 8·3 | 153·7 | 384·5 | 38·6 |
| 12:09 to 12:39 | 16·5 | 35·7 | 53·2 | 60·5 | 8·3 | 153·7 | 384·5 | 38·9 |
| P. M. | | | | | | | | |
| 1:58 to 2:28 | 17·4 | 35·8 | 53·2 | 60·5 | 7·3 | 135·2 | 338·1 | 38·9 |
| 2:55 to 3:25 | 17·0 | 35·7 | 52·7 | 60·5 | 7·8 | 144·5 | 361·3 | 38·7 |
| 3:49 to 4:19 | 16·1 | 35·5 | 51·6 | 60·5 | 8·9 | 164·9 | 412·3 | 38·9 |
| 4:48 to 5:18 | 16·0 | 35·3 | 51·3 | 60·5 | 9·2 | 170·4 | 423·7 | 38·8 |
| Average, | 16·7 | 35·6 | 52·3 | 60·5 | 8·2 | 151·3 | 378·4 | 38·8 |
| March 27. | | | | | | | | |
| A. M. | | | | | | | | |
| 8:33 to 9:03 | 16·2 | 35·6 | 51·8 | 60·5 | 8·7 | 161·1 | 403·0 | 38·9 |
| 9:30 to 10:00 | 15·7 | 35·5 | 51·2 | 60·5 | 9·3 | 172·3 | 430·8 | 38·9 |

Following is the average daily excretion of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature:

| | | | |
|---|---|---|---|
| March 24. | 38·0° C. | 381·0 milligrams $CO_2$ | |
| " 25. | 38·4 | 363·7 " | " |
| " 26. | 38·8 | 378·4 " | " |
| " 27. | 38·9 | 416·9 " | " |

The morphine was introduced into the stomach in solution in the following amounts:

| | | | | |
|---|---|---|---|---|
| March 24. | 6:00 p. m. | 0·075 gram morphine sulphate. | | |
| " 25. | 8:35 a. m. | 0·075 | " | " |
| " 25. | 12:35 p. m. | 0·075 | " | " |
| " 25. | 5:50 p. m. | 0·100 | " | " |
| " 26. | 9:15 a. m. | 0·100 | " | " |
| " 26. | 12:50 p. m. | 0·100 | " | " |
| | | 0·525 | | |

large dose of morphine sulphate was given, and given by hypodermic injection. In this case, as before, there was no increased muscular activity, but there was a sudden and rapid fall both in temperature and in the amount of carbonic acid excreted, the latter of which was still quite pronounced on the following day, although the temperature has gone back to normal. This diminution in carbonic acid was accompanied by a profound narcotism, the animal lying almost motionless and with a respiration ranging from 8 to 20 per minute. The results, which are to a certain extent corroborative of Boeck and Bauer's, are shown in the accompanying table:

SECOND SERIES OF EXPERIMENTS WITH MORPHINE.

*Normal period without morphine sulphate.*

| Time. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| March 12. A. M. 9:12 to 9:42 | 15.4 | 34·1 | 49·5 | 59·25 | 9·75 | 180·6 | 465·0 | 39·1 |
| 10:00 A. M., injected subcutaneously 0·100 gram morphine sulphate. | | | | | | | | |
| A. M. 10:23 to 10:53 | 16·9 | 34·5 | 51·4 | 59·25 | 7·85 | 145·4 | 363·5 | 36·6 |
| 11:29 to 11:59 | 17·3 | 34·5 | 51·8 | 59·25 | 7·45 | 138·0 | 342·5 | 34·4 |
| 12:32 to 1:02 | 16·3 | 34·1 | 50·4 | 59·25 | 8·85 | 164·0 | 410·0 | 33·3 |
| P. M. 2:48 to 3:18 | 17·0 | 34·2 | 51·2 | 59·25 | 8·05 | 149·2 | 373·0 | 34·4 |
| 3:49 to 4:19 | 17·8 | 34·6 | 52·4 | 59·25 | 6·85 | 126·9 | 317·3 | 35·0 |
| 4:47 to 5:17 | 17·1 | 34·3 | 51·4 | 59·25 | 7·85 | 145·4 | 363·5 | 36·3 |
| March 13. 10:08 to 10:38 | 16·9 | 34·3 | 51·2 | 59·25 | 8·05 | 149·2 | 373·0 | 39·2 |

*Action of quinine sulphate.*

The importance of quinine as a therapeutic agent and particularly its value as a febrifuge has led to a thorough study of its physiolog-

ical action. In this connection, the action of quinine on proteid metabolism has been very thoroughly investigated, but as to its exact influence on the decomposition of non-nitrogenous matter, as shown by its effects on the elimination of carbonic acid, there is less unanimity of opinion. This is naturally a point of considerable importance for if, as is generally supposed, the alkaloid has the power of diminishing body temperature, it would presumably be due to its influence on the combustion of non-nitrogenous matter in the body. Ranke, Kerner, von Boeck and others have plainly shown the power of quinine to diminish proteid metabolism, but Strassburg, by an elaborate series of experiments* found that the alkaloid had no very decided effect upon the elimination of carbonic acid, either in healthy or fevered rabbits.

Boeck and Bauer,† however, from experiments on cats, claim that quinine in the first stage of its action diminishes somewhat the production of carbonic acid, owing to its inhibitory action on the tissue cells; but when large doses of quinine are given, so that convulsions appear, then there is an increased production of carbonic acid, owing to the greater decomposition of non-nitrogenous matter incident to increased muscular activity. With small doses of the alkaloid, it is to be presumed that the slight diminution in carbonic acid noticed by Boeck and Bauer comes simply from diminished proteid metabolism.

In our experiments, rabbits only were used and these in a condition of hunger, having been deprived of food for three days prior to the experiment. In the first series of experiments, the total amount of quinine given was quite large, so that at last the animal finally died from its effects. No decided action on the production of carbonic acid was noticed until just before the animal's death, when both the body temperature and the amount of carbonic acid fell quite noticeably. On the second day of the experiment, when the quinine was first being given, the body temperature, as taken per rectum, fell quite gradually until it finally reached a point 1·5 °C. below the average of the normal period. The results of the experiment are to be seen in the accompanying tables. The quinine given was in the form of hard, gelatin-coated pills and possibly was not as rapidly absorbed as might otherwise have been. At no time was the rabbit in convulsions.

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* Quoted from Dr. H. C. Wood, Therapeutics, p. 75.

† Zeitschrift für Biologie, Band x, p. 350.

## First Series of Experiments with Quinine.

*Normal period, without quinine sulphate.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| April 12. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 9:11 to 9:41 | 9·5 | 24·2 | 33·7 | 41·5 | 7·8 | 144·5 | 361·5 | 38·7 |
| 10:05 to 10:35 | 10·8 | 24·4 | 35·2 | 41·5 | 6·3 | 116·7 | 291·8 | 38·7 |
| 10:58 to 11:28 | 10·8 | 24·2 | 35·0 | 41·5 | 6·5 | 120·4 | 301·1 | 38·7 |
| 11:54 to 12:24 | 10·7 | 24·2 | 34·9 | 41·5 | 6·6 | 122·2 | 305·7 | 38·3 |
| P. M. | | | | | | | | |
| 1:58 to 2:28 | 10·0 | 24·0 | 34·0 | 41·5 | 7·5 | 138·9 | 347·4 | 38·6 |
| 2:51 to 3:21 | 10·0 | 24·2 | 34·2 | 41·5 | 7·3 | 135·2 | 338·1 | 38·4 |
| 3:49 to 4:19 | 10·5 | 24·3 | 34·8 | 41·5 | 6·7 | 124·1 | 310·4 | 38·7 |
| 4:41 to 5:11 | 11·0 | 24·3 | 35·3 | 41·5 | 6·2 | 114·8 | 287·2 | 38·8 |
| Average, | 10·4 | 24·2 | 34·6 | 41·5 | 6·8 | 127·1 | 317·8 | 38·6 |
| April 13. | | *With quinine sulphate.* | | | | | | |
| A. M. | | | | | | | | |
| 9:14 to 9:44 | 9·3 | 23·9 | 33·2 | 41·5 | 8·3 | 153·7 | 384·5 | 38·6 |
| 10:10 to 10:40 | 11·3 | 23·9 | 35·2 | 41·5 | 6·3 | 116·7 | 291·8 | 38·6 |
| 11:05 to 11:35 | 11·0 | 24·4 | 35·4 | 41·5 | 6·1 | 113·0 | 282·6 | 38·2 |
| 11:58 to 12:28 | 10·3 | 24·2 | 34·5 | 41·5 | 7·0 | 129·7 | 314·3 | 38·2 |
| P. M. | | | | | | | | |
| 1:55 to 2:25 | 9·4 | 24·0 | 33·4 | 41·5 | 8·1 | 150·0 | 375·2 | 38·1 |
| 2:46 to 3:16 | 10·1 | 24·2 | 34·3 | 41·5 | 7·2 | 133·4 | 333·3 | 37·6 |
| 3:40 to 4:10 | 9·3 | 23·8 | 33·1 | 41·5 | 8·4 | 168·7 | 340·5 | 37·1 |
| 4:32 to 5:02 | 10·3 | 24·1 | 34·4 | 41·5 | 7·1 | 143·5 | 282·6 | 38·0 |
| Average, | 10·1 | 24·1 | 34·2 | 41·5 | 7·3 | 138·6 | 325·6 | 38·1 |

*With quinine sulphate—continued.*

| Date. April 14. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 8:54 to 9:24 | 10·0 | 24·3 | 34·3 | 41·5 | 7·2 | 133·4 | 333·5 | 38·6 |
| 9:50 to 10:20 | 10·2 | 24·1 | 34·3 | 41·5 | 7·2 | 133·4 | 333·5 | 38·2 |
| 10:43 to 11:13 | 9·8 | 24·0 | 33·8 | 41·5 | 7·7 | 142·6 | 356·7 | 38·3 |
| 11:38 to 12:08 | 10·9 | 24·3 | 35·2 | 41·5 | 6·3 | 116·7 | 291·8 | 37·6 |
| P. M. 1:57 to 2:27 | 11·3 | 24·5 | 35·8 | 41·5 | 5·7 | 105·6 | 264·0 | 34·7 |
| Average, | 10·4 | 24·2 | 34·6 | 41.5 | 6·9 | 126·3 | 315·9 | 37·5 |

The following amounts of quinine were given by way of the mouth:

| | | | | | |
|---|---|---|---|---|---|
| April 12 | 5:20 p. m. | 0·130 | gram | quinine | sulphate. |
| " 13 | 9:10 a. m. | 0·260 | " | " | " |
| " 13 | 1:00 p. m. | 0·390 | " | " | " |
| " 13 | 5:15 p. m. | 0·520 | " | " | " |
| " 14 | 8:45 a. m. | 0·520 | " | " | " |
| " 14 | 12:00 m. | 0·780 | " | " | " |
| | | 2·600 | | | |

Rabbit died at 3 p. m. April 14.

Following are the average daily results in temperature and in the amount of carbonic acid excreted per 37·5 litres of aspirated air.

| | | | | |
|---|---|---|---|---|
| April 12 | 38·6° C. | 317·8 | milligrams | $CO_2$. |
| " 13 | 38·1 " | 325·6 | " | " |
| " 14 | 37·5 " | 315·9 | " | " |

SECOND SERIES OF EXPERIMENTS WITH QUININE.

*Normal period, without quinine sulphate.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| May 17. | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| A. M. | | | | | | | | |
| 8:40 to 9:10 | 5·3 | 22·2 | 27·5 | 41·8 | 14·3 | 289·0 | 722·7 | 38·8 |
| 9:33 to 10:03 | 6·1 | 22·8 | 28·9 | 41·8 | 12·9 | 260·7 | 651·9 | 38·8 |
| 10:29 to 10:59 | 6·2 | 22·6 | 28·8 | 41·8 | 13·0 | 262·7 | 657·0 | 38·8 |
| 11:24 to 11:54 | 4·9 | 21·9 | 26·8 | 41·8 | 15·0 | 303·2 | 758·1 | 39·1 |
| P. M. | | | | | | | | |
| 1:55 to 2:25 | 5·0 | 22·1 | 27·1 | 41·8 | 14·7 | 297·1 | 742·9 | 39·0 |
| 2:48 to 3:18 | 4·5 | 22·0 | 26·5 | 41·8 | 15·3 | 309·2 | 773·2 | 38·9 |
| 3:43 to 4:13 | 6·7 | 23·1 | 29·8 | 41·8 | 12·0 | 242·5 | 606·5 | 38·8 |
| 4:35 to 5:05 | 6·7 | 23·0 | 29·7 | 41·8 | 12·1 | 244·5 | 611·5 | 38·9 |
| Average, | 5·7 | 22·4 | 28·1 | 41·8 | 13·7 | 276·8 | 690·5 | 38·6 |
| May 18. | | *With quinine sulphate.* | | | | | | |
| A. M. | | | | | | | | |
| 9:05 to 9:35 | 6·2 | 22·5 | 28·7 | 41·8 | 13·1 | 264·8 | 662·0 | 38·6 |
| 10:00 to 11:30 | 7·5 | 23·0 | 30·5 | 41·8 | 11·3 | 228·4 | 571·1 | 38·4 |
| 10:51 to 11:21 | 5·6 | 22·1 | 27·7 | 41·8 | 14·1 | 285·0 | 712·6 | 38·3 |
| 11:46 to 12:16 | 6·0 | 22·5 | 28·5 | 41·8 | 13·3 | 268·8 | 672·2 | 38·8 |
| P. M. | | | | | | | | |
| 2:14 to 2:44 | 6·5 | 22·8 | 29·3 | 41·8 | 12·5 | 252·6 | 631·7 | 38·9 |
| 3:07 to 3:37 | 6·5 | 22·9 | 29·4 | 41·8 | 12·4 | 249·6 | 624·1 | 39·0 |
| 4:01 to 4:31 | 7·0 | 23·1 | 30·1 | 41·8 | 11·7 | 236·5 | 591·3 | 39·0 |
| 4:58 to 5:28 | 6·0 | 22·1 | 28·1 | 41·8 | 13·7 | 276·9 | 692·4 | 38·4 |
| Average, | 6·4 | 22·6 | 29·0 | 41·8 | 12·8 | 257·8 | 644·7 | 38·7 |

***With quinine sulphate—continued.***

| Date. May 19. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. | | | | | | | | |
| 9:02 to 9:32 | 7·3 | 23·0 | 30·3 | 41·8 | 11·5 | 232·4 | 581·2 | 38·9 |
| 9:57 to 10:27 | 7·6 | 23·2 | 30·8 | 41·8 | 11·0 | 222·8 | 555·9 | 38·4 |
| 10:50 to 11:20 | 7·2 | 23·1 | 30·3 | 41·8 | 11·5 | 232·4 | 581·2 | 38·7 |
| 11:43 to 12:13 | 6·9 | 23·0 | 29·9 | 41·8 | 11·9 | 240·5 | 601·4 | 38·6 |
| P. M. | | | | | | | | |
| 2:03 to 2:33 | 7·5 | 23·2 | 30·7 | 41·8 | 11·1 | 223·3 | 558·4 | 38·9 |
| 2:54 to 3:24 | 8·2 | 23·6 | 31·8 | 41·8 | 10·0 | 202·1 | 505·4 | 38·5 |
| 3:47 to 4:17 | 7·4 | 23·3 | 30·7 | 41·8 | 11·1 | 224·3 | 561·0 | 38·8 |
| 4:40 to 5:10 | 7·1 | 23·1 | 30·2 | 41·8 | 11·6 | 234·5 | 586·3 | 38·8 |
| Average, | 7·4 | 23·2 | 30·6 | 41·8 | 11·2 | 226·4 | 566·4 | 38·7 |

The quinine was given by way of the mouth in gelatin capsules, in the following quantities:

| | | | |
|---|---|---|---|
| May 17 | 5:30 p. m. | 0·250 | gram quinine sulphate. |
| " 18 | 8:50 a. m. | 0·250 | " " " |
| " 18 | 12:30 p. m. | 0·250 | " " " |
| " 18 | 5:35 p. m. | 0·250 | " " " |
| " 19 | 8:50 a. m. | 0·325 | " " " |
| " 19 | 1:55 p. m. | 0·250 | " " " |
| | | 1·575 | |

Following are the average daily excretions of carbonic acid expressed in milligrams of $CO_2$ per 37·5 litres of aspirated air, together with the average body temperature:

| | | | |
|---|---|---|---|
| May 17 | 38·6° C. | 690·5 | milligrams $CO_2$ |
| " 18 | 38·7 " | 644·7 | " " |
| " 19 | 38·7 " | 566·4 | " " |

### Third Series of Experiments with Quinine.

*Normal period, without quinine sulphate.*

| Time. March 8. | Oxalic acid to neutralize baryta solution. tube a. c. c. | Oxalic acid to neutralize baryta solution. tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 9:50 to 10:20 | 15·5 | 33·9 | 49·4 | 59·25 | 9·85 | 182·5 | 456·3 | 39·2 |
| 10:58 A. M. injected subcutaneously 0·083 gram quinine sulphate. | | | | | | | | |
| 11:15 to 11:45 | 16·9 | 34·5 | 51·4 | 59·25 | 7·85 | 145·4 | 363·5 | 38·4 |
| 12:25 to 12:55 | 15·3 | 33·9 | 49·2 | 59·25 | 10·05 | 186·2 | 465·5 | 39·9 |
| P. M. 3:00 to 3:30 | 15·6 | 34·0 | 49·6 | 59·25 | 9·65 | 178·8 | 447·0 | 39·8 |
| 4:06 to 4:36 | 15·8 | 33·7 | 49·5 | 59·25 | 9·75 | 180·7 | 451·8 | 39·9 |
| Average, | 15·9 | 34·0 | 49·9 | 59·25 | 9·35 | 172·8 | 432·0 | 39·5 |

### Fourth Series of Experiments with Quinine.

*Normal period, without quinine sulphate.*

| Time. March 10. | Oxalic acid to neutralize baryta solution. tube a. c. c. | Oxalic acid to neutralize baryta solution. tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature ° C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 9:11 to 9:41 | 15·0 | 33·8 | 48·8 | 59·25 | 10·45 | 193·6 | 484·0 | 38·8 |
| 10:15 A. M. injected subcutaneously 0·15 gram quinine sulphate. | | | | | | | | |
| 10:33 to 11:03 | 16·5 | 34·6 | 51·1 | 59·25 | 8·15 | 151·0 | 377·5 | 38·4 |
| 11:39 to 12:09 | 15·4 | 33·9 | 49·3 | 59·25 | 9·95 | 184·4 | 461·0 | 38·9 |
| 12:38 to 1:08 | 15·3 | 34·1 | 49·4 | 59·25 | 9·85 | 182·5 | 456·3 | 38·4 |
| P. M. 2:54 to 3:24 | 16·0 | 34·3 | 50·3 | 59·25 | 8·95 | 165·8 | 414·5 | 39·1 |
| 4:07 to 4:37 | 15·9 | 34·1 | 50·0 | 59·25 | 9·25 | 171·4 | 428·5 | 38·6 |
| Average, | 15·8 | 34·2 | 50·0 | 59·25 | 9·25 | 171·0 | 427·6 | 38·7 |

EXPERIMENT WITH CINCHONIDINE.

*Normal period, without cinchonidine sulphate.*

| Date. June 24. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37 5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. | | | | | | | | |
| 9:00 to 9:30 | 10·6 | 28·8 | 39·4 | 50·2 | 10·8 | 218·3 | 545·8 | 36·4 |
| 9:56 to 10·26 | 11·2 | 29·1 | 40·3 | 50·2 | 9·9 | 200·1 | 500·3 | 37·4 |
| 10:47 to 11:17 | 12·0 | 29·0 | 41·0 | 50·2 | 9·2 | 185·9 | 465·0 | 38·1 |
| 11:38 to 12:08 | 10·6 | 28·7 | 39·3 | 50·2 | 10·9 | 220·3 | 550·9 | 36·3 |
| P. M. | | | | | | | | |
| 2:03 to 2:33 | 10·3 | 28·5 | 38·8 | 50·2 | 11·4 | 230·4 | 576·1 | 36·9 |
| 2:51 to 3:21 | 9·4 | 28·2 | 37·6 | 50·2 | 12·6 | 254·9 | 636·8 | 37·1 |
| 3:42 to 4:12 | 9·7 | 28·5 | 38·2 | 50·2 | 12·0 | 242·5 | 606·5 | 37·0 |
| 4:30 to 5:00 | 9·7 | 28·5 | 38·2 | 50·2 | 12·0 | 242·5 | 606·5 | 36·4 |
| Average, | 10·5 | 28·6 | 39·1 | 50·2 | 11·1 | 224·3 | 561·0 | 37·0 |
| June 25. | | *With cinchonidine sulphate.* | | | | | | |
| A. M. | | | | | | | | |
| 9:03 to 9:33 | 10·8 | 28·6 | 39·4 | 50·2 | 10·8 | 218·3 | 545·8 | 38·6 |
| 9:58 to 10:28 | 11·4 | 28·9 | 40·3 | 50·2 | 9·9 | 200·1 | 500·3 | 38·6 |
| 10:53 to 11:23 | 11·1 | 28·7 | 39·8 | 50·2 | 10·4 | 210·2 | 525·6 | 38·6 |
| 11:48 to 12:18 | 11·6 | 28·7 | 40·3 | 50·2 | 9·9 | 200·1 | 500·3 | 38·7 |
| P. M. | | | | | | | | |
| 2:04 to 2:34 | 11·1 | 28·8 | 39·9 | 50·2 | 10·3 | 208·2 | 520·5 | 38·9 |
| 2:59 to 3:29 | 11·5 | 29·0 | 40·5 | 50·2 | 9·7 | 196·0 | 490·2 | 39·0 |
| 3:56 to 4:26 | 11·6 | 29·0 | 40·6 | 50·2 | 9·6 | 194·0 | 485·2 | 39·0 |
| 4:50 to 5:20 | 12·1 | 29·1 | 41·2 | 50·2 | 9·0 | 181·9 | 454·8 | 39·1 |
| Average, | 11·4 | 28·8 | 40·2 | 50·2 | 10·0 | 201·1 | 502·8 | 38·8 |

Following are the average daily amounts of carbonic acid excreted, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature.

| | | | |
|---|---|---|---|
| June 24, | 37·0° C., | 561·0 | milligrams $CO_2$. |
| " 25, | 38·8 | 502·8 | " " |

Following are the doses of cinchonidine :

| | | | |
|---|---|---|---|
| June 24, | 5:12 P. M., | 1·000 | gram cinchonidine sulphate. |
| " 25, | 10:35 A. M., | 0·250 | " " " |
| " 25, | 12:35 P. M., | 0·325 | " " " |
| | | 1·575 | |

Rabbit died at 5:40 P. M., June 25, in convulsions.

In the second series of experiments, smaller doses of quinine were employed, so that no special toxic action was observed. In this case there seemed to be a gradual falling off in the amount of carbonic acid produced; such a decrease as might be assumed would naturally result from diminished proteid metabolism.

The results are shown in the accompanying tables.

The body temperature, as determined per rectum did not show any change whatever under the influence of this quantity of quinine (total dose 1·575 gram of quinine sulphate). There does not appear to be any proof that moderate doses of quinine lower the body temperature of healthy animals or even man, and Kerner found in his experiments that a full dose of quinine given to a healthy man would prevent the usual rise of temperature resulting from vigorous exercise, but did not affect the temperature under ordinary circumstances. Liebermeister* has also reported that the alkaloid has no constant depressing action on the bodily heat in health.

Two other short series of experiments were tried with quinine, also with rabbits in a condition of hunger. In both of these cases the quinine was introduced by sub-cutaneous injection in the form of sulphate. In both cases there was a slight fall in temperature, accompanied with a noticeable decrease in the amount of carbonic acid eliminated, directly after injection of the quinine. This effect, however, was only temporary, for the temperature quickly rose to the normal, and even somewhat above the normal point, while the carbonic acid in the third series came quite back to the normal and in the fourth series remained only a little way below.

It would appear, therefore, from our experiments, that in a healthy, hungry rabbit moderate doses of quinine sulphate exercise at the most only a very slight depressing influence on body temperature, and have but a minimum effect on the production of carbonic acid.

### *Action of cinchonidine sulphate.*

Previous experiments † on man have shown that cinchonidine has the power of lessening materially the elimination of nitrogen, presumably through its inhibitory action on proteid metabolism.

Cinchonidine is supposed to have much the same physiological action as quinine and cinchonine, only weaker. Our present experi-

* Deutsch. Archiv für Klinische Medicin, Band iii.

† See Chittenden and Whitehouse, Studies from the Laboratory of Physiological Chemistry, vol. i, p. 164.

ments with the alkaloid, using quite large amounts of the sulphate, show a somewhat different action from quinine. Using a large rabbit, without food for three days, and giving it by way of the mouth, in gelatin capsules, large doses of the alkaloid, there was a very noticeable and constant rise in temperature up to the very time of death, accompanied by a slight but gradual diminution in the amount of carbonic acid given off. In all, 1·575 grams of cinchonidine sulphate were given; an amount exactly equal to the quinine sulphate given in the second series of experiments with quinine. With cinchonidine, however, the rabbit was much prostrated, showed symptoms of tetanic convulsions, and finally died in a vigorous tetanic spasm at the end of the second day.

The results are shown in the preceding table.

### *Action of Antipyrine.*

Antipyrine or dimethyloxychinicine has of late been much experimented with. Among the many statements which we have seen recently concerning its action are the following, which are of interest in this connection. Arduin* found that 3 grams given to a rabbit produced cataleptic stiffness, diminished reflexes, etc., followed by violent convulsions. There was also a very marked fall of bodily temperature. Anseroff,† by experiments on animals, found that the alkaloid caused an increase of blood pressure and a decrease of internal temperature, as shown by a thermometer in the rectum, but a considerable rise in the external temperature, sometimes as much as 12° C. Pavlinoff‡ has reported that antipyrine produces a very considerable quickening of the respiration, while Dr. Walter, of St. Petersburg,§ is reported as having found that the alkaloid while reducing febrile temperature, also reduces nitrogenous tissue changes; and further, that the assimilation of proteids is materially favored by the drug. F. Müller‖ has also found that in fever antipyrine diminishes the excretion of nitrogen. Coppola,¶ however, states that in the case of a dog, 0·3–0·4 gram of antipyrine was wholly without action on its excretion of nitrogen. Coppola has further found that the

* Abstract in Therapeutic Gazette, 3d series, vol. i, p. 677.

† Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 315.

‡ Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 339.

§ Abstract in Therapeutic Gazette. 3d series, vol. ii, p. 53.

‖ Jahresbericht für Thierchemie, xiv, 242.

¶ Jahresbericht für Thierchemie, xv, 97.

FIRST SERIES OF EXPERIMENTS WITH ANTIPYRINE.

*Normal period, without antipyrine.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| | tube a. c. c. | tubes b and c. c. c. | | | | | | |
| May 24. | | | | | | | | |
| A. M. 8:54 to 9:24 | 8·6 | 26·4 | 35·0 | 46·1 | 11·1 | 224·3 | 561·0 | 38·4 |
| 9:52 to 10:22 | 8·8 | 26·5 | 35·3 | 46·1 | 10·8 | 218·3 | 545·8 | 38·4 |
| 10:47 to 11:17 | 9·5 | 26·4 | 35·9 | 46·1 | 10·2 | 205·1 | 513·0 | 38·4 |
| P. M. 1:55 to 2:25 | 9·1 | 26·3 | 35·4 | 46·1 | 10·7 | 216·3 | 540·8 | 38·3 |
| 2:47 to 3:17 | 9·5 | 26·4 | 35·9 | 46·1 | 10·2 | 205·1 | 513·0 | 38·6 |
| 3:40 to 4:10 | 9·1 | 26·5 | 35·6 | 46·1 | 10·5 | 212·2 | 530·6 | 38·4 |
| 4:35 to 5:05 | 8·8 | 26·3 | 35·1 | 46·1 | 11·0 | 222·3 | 555·9 | 38·4 |
| Average, | 9·0 | 26·4 | 35·4 | 46·1 | 10·7 | 214·8 | 535·7 | 38·4 |
| May 25. | | | *With antipyrine.* | | | | | |
| A. M. 8:56 to 9:26 | 9·6 | 26·4 | 36·0 | 46·1 | 10·1 | 204·1 | 510·4 | 38·2 |
| 9:53 to 10:23 | 9·6 | 26·4 | 36·0 | 46·1 | 10·1 | 204·1 | 510·4 | 38·1 |
| 10:49 to 11:19 | 7·5 | 25·6 | 33·1 | 46·1 | 13·0 | 262·7 | 657·0 | 38·0 |
| 11:47 to 12:17 | 8·4 | 25·9 | 34·3 | 46·1 | 11·8 | 238·5 | 596·3 | 38·1 |
| P. M. 2:15 to 2:45 | 7·9 | 26·0 | 33·9 | 46·1 | 12·2 | 246·6 | 616·6 | 38·1 |
| 3:15 to 3:45 | 8·7 | 25·8 | 34·5 | 46·1 | 11·6 | 234·5 | 586·3 | 38·3 |
| 4:15 to 4:45 | 8·4 | 26·0 | 34·4 | 46·1 | 11·7 | 236·5 | 591·3 | 38·0 |
| 5:09 to 5:39 | 7·4 | 25·6 | 33·0 | 46·1 | 13·1 | 264·8 | 662·0 | 38·1 |
| Average, | 8·5 | 25·9 | 34·4 | 46·1 | 11·7 | 236·5 | 591·3 | 38·1 |
| May 26. | | | | | | | | |
| A. M. 9:02 to 9:32 | 11·3 | 26·8 | 38·1 | 46·1 | 8·0 | 161·7 | 404·3 | 36·2 |
| 9:59 to 10:29 | 9·4 | 26·0 | 35·4 | 46·1 | 10·7 | 216·3 | 540·8 | 35·3 |

alkaloid not only has a noticeable antipyretic action in conditions of fever, but also reduces the temperature in healthy organisms. The extent of reduction, however, is not great, ranging only from 0·1 to 0·6 of a degree. Further, the diminution in temperature is to be ascribed, according to Coppola, not to diminished metabolic activity, but to increased giving up of heat, due to dilatation of the blood-vessels by the antipyrine. Jacubowitsch * also claims for antipyrine an inhibitory action on the excretion of uric acid. So far as our knowledge extends, however, no experiments have been tried as to the influence of this therapeutic agent on the production of carbonic acid.

Our experiments have been confined wholly to rabbits, and those in a condition of hunger. In the first series of experiments, during the antipyrine period, the alkaloid was given in large and oft-repeated doses, in the form of powder, in gelatin capsules, at follows:

| | | | | |
|---|---|---|---|---|
| May 25, | 8:33 A. M., | 0·2 | gram | antipyrine. |
| " 25, | 9:35 " | 0·2 | " | " |
| " 25, | 10:30 " | 0·2 | " | " |
| " 25, | 11:27 " | 0·2 | " | " |
| " 25, | 12:27 P. M., | 0·6 | " | " |
| " 25, | 2:55 " | 0·6 | " | " |
| " 25, | 3:54 " | 0·6 | " | " |
| " 25, | 4:51 " | 0·6 | " | " |
| " 25, | 5:46 " | 0·6 | " | " |
| " 26, | 8:45 A. M., | 0·6 | " | " |
| " 26, | 9:38 " | 1·0 | " | " |
| | | 5·4 | | |

The accompanying tables show the results obtained. At the end of the last period of the second day (May 26) respiration was very rapid, about 208 per minute, and during that period the amount of carbonic acid excreted was larger than in any other. In spite of the large dose taken, however, the alkaloid appears to have had no special action on the production of carbonic acid, and further, the temperature was only very slightly lowered until on the last day, just prior to the animal's death. On the last day of the experiment (May 26) the rabbit appeared much prostrated, and at 10.35 A. M. was seized with a convulsion, followed soon after by three others, lying at 11.30 A. M.

In the second series of experiments somewhat smaller amounts of antipyrine were given, but here, as in the first series, there was no

* Jahresbericht für Thierchemie, xv, p. 444.

SECOND SERIES OF EXPERIMENTS WITH ANTIPYRINE.

*Normal period, without antipyrine.*

| Date. | Oxalic acid to neutralize baryta solution. | | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. ° C. |
|---|---|---|---|---|---|---|---|---|
| May 31. | tube a. c. c | tubes b and c. c. c. | | | | | | |
| A. M. 9:02 to 9:32 | 8·5 | 25·3 | 33·8 | 44.3 | 10·5 | 211·2 | 528·1 | 38·9 |
| 9:57 to 10:27 | 8·1 | 25·1 | 33·2 | 44·3 | 11·1 | 224·3 | 561·0 | 38·9 |
| 10:59 to 11:29 | 9·1 | 25·5 | 34·6 | 44·3 | 9·7 | 196·0 | 490·2 | 38·9 |
| 11:51 to 12:21 | 9·3 | 25·5 | 34·8 | 44·3 | 9·5 | 191·0 | 477·6 | 38·9 |
| P. M. 1:44 to 2:14 | 9·8 | 25·7 | 35·5 | 44·3 | 8·8 | 176·8 | 442·2 | 38·7 |
| 3:30 to 4:00 | 9·3 | 25·5 | 34·8 | 44·3 | 9·5 | 192·0 | 480·1 | 38·9 |
| 4:21 to 4:51 | 9·7 | 25·7 | 35·4 | 44·3 | 8·9 | 179·9 | 449·8 | 38·6 |
| Average, | 9·1 | 25·5 | 34·6 | 44·3 | 9·7 | 195·9 | 489·9 | 38·8 |
| June 1. | | | *With antipyrine.* | | | | | |
| A. M. 8:53 to 9:23 | 9·9 | 25·5 | 35·4 | 44·3 | 8·9 | 179·9 | 449·8 | 38·7 |
| 9:50 to 10:20 | 9·4 | 25·5 | 34·9 | 44·3 | 9·4 | 190·0 | 475·1 | 38·9 |
| 10:43 to 11:13 | 10·1 | 25·7 | 35·8 | 44·3 | 8·5 | 170·8 | 427·0 | 38·9 |
| 11:40 to 12:10 | 9·7 | 25·7 | 35·4 | 44·3 | 8·9 | 179·9 | 449·8 | 38·7 |
| P. M. 2:15 to 2:45 | 9·6 | 25·6 | 35·2 | 44·3 | 9·1 | 183·9 | 459·9 | 39·2 |
| 3:12 to 3:42 | 9·9 | 25·8 | 35·7 | 44·3 | 8·6 | 173·8 | 434·6 | 38·4 |
| 4:11 to 4:41 | 9·7 | 25·6 | 35·3 | 44·3 | 9·0 | 181·9 | 454·8 | 38·3 |
| 5:05 to 5:35 | 8·7 | 25·3 | 34·0 | 44·3 | 10·3 | 208·2 | 520·5 | 38·4 |
| Average, | 9·6 | 25·6 | 35·2 | 44·3 | 9.08 | 183·5 | 458·9 | 38·7 |

*With antipyrine—continued.*

| Date. June 2. | Oxalic acid to neutralize baryta solution. tube a. c. c. | tubes b and c. c. c. | Total oxalic acid used. c. c. | Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c. | Difference. c. c. oxalic acid. | $CO_2$ in a, b and c. mg. | $CO_2$ in 37·5 L. aspirated air. mg. | Body temperature. °C. |
|---|---|---|---|---|---|---|---|---|
| A. M. 8:56 to 9:26 | 10·0 | 25·5 | 35·5 | 44·3 | 8·8 | 177·8 | 444·7 | 38·7 |
| 9:53 to 10:23 | 9·8 | 25·6 | 35·4 | 44·3 | 8·9 | 179·9 | 449·8 | 38·6 |
| 10:47 to 11:17 | 8·8 | 25·3 | 34·1 | 44·3 | 10·2 | 206·1 | 515·5 | 37·8 |
| 11:43 to 12:13 | 8·6 | 25·1 | 33·7 | 44·3 | 10·6 | 214·2 | 535·7 | 37·1 |
| P. M. 2:02 to 2:32 | 8·6 | 25·1 | 33·7 | 44·3 | 10·6 | 213·2 | 533·2 | 37·1 |
| 2:55 to 3:25 | 8·7 | 25·1 | 33·8 | 44·3 | 10·5 | 212·2 | 530·6 | 37·3 |
| 3:54 to 4:24 | 8·8 | 25·3 | 34·1 | 44·3 | 10·2 | 206·1 | 515·5 | 37·6 |
| 4:47 to 5:17 | 8·5 | 25·1 | 33·6 | 44·3 | 10·7 | 216·3 | 540·8 | 37·5 |
| Average, | 8·9 | 25·3 | 34·2 | 44·3 | 10·06 | 208·2 | 508·2 | 37·7 |

Following are the average daily excretions of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with average daily body temperature:

| | | |
|---|---|---|
| May 31 | 38·8° C. | 489·9 milligrams $CO_2$. |
| June 1 | 38·7 | 458·9 " " |
| " 2 | 37·7 | 508·2 " " |

Antipyrine was given in the following quantities by mouth, in gelatin capsules:

| | | |
|---|---|---|
| May 31 | 4.57 p. m. | 0·2 gram antipyrine. |
| June 1 | 8:40 a. m. | 0·2 " |
| " 1 | 9:31 " | 0·2 " |
| " 1 | 10:25 " | 0·2 " |
| " 1 | 11:20 " | 0·2 " |
| " 1 | 12:17 p. m. | 0·2 " |
| " 1 | 2:52 " | 0·2 " |
| " 1 | 3:49 " | 0·2 " |
| " 1 | 4:48 " | 0·2 " |
| " 1 | 5:40 " | 0·2 " |
| " 2 | 8:45 a. m. | 0·5 " |
| " 2 | 9:34 " | 0·5 " |
| " 2 | 10:31 " | 0·5 " |
| " 2 | 11:24 " | 0·5 " |
| " 2 | 4:45 p. m. | 0·2 " |
| | | 4·2 |

discernible action on the excretion of carbonic acid. Further, the alkaloid did not noticeably lower the body temperature until toward the close of the second day, when the quantity given had reached an amount nearly sufficient to produce a fatal result. We have then to conclude that antipyrine, at least in therapeutic doses, has no special influence on the production or elimination of carbonic acid by the rabbit.

# XXIV.—New England Spiders of the Family Ciniflonidæ.

By J. H. Emerton.

The spinning organs of the Ciniflonidæ differ from those of all other spiders. They have in front of the usual spinnerets an additional spinning organ, the *cribellum*, with spinning tubes like the other spinnerets, but much finer, and they have on the metatarsus of each hind leg a row of stiff hairs, the *calamistrum*, by which the thread is combed from the cribellum in a loose curly band. This band of loose thread forms part of every cobweb made by these spiders (Pl. x, fig. 1*g*.) and is easily distinguished in new webs by its width and white color and in old webs by the amount of dust which it collects.

The feet have three claws and some species have a few curved and toothed spines under the claws, like *Epeiridæ* and *Theridiidæ*. The tracheæ are large and open in a wide slit in front of the cribellum.

The colors are generally dull brown and grey. A double row of oblique light markings on the back of the abdomen, which shows most distinctly in *Amaurobius*, is in a modified from the usual marking of the abdomen throughout the family, often varying greatly in shape in the same species.

These spiders were first placed together in one family by Black wall, who in 1839 noticed their peculiar webs and spinning organs. Before that time they had been scattered among various families according to their size, form, and habits. They have been treated in the same way by Thorell in his book on the genera of European spiders, and by Menge in the spiders of Prussia. Simon divides the French species into two families, *Dictynidæ* and *Uloboridæ*. Bertkau in his latest revision of the family, in 1882, carries the division into families still further and unites them all into a sub-order, *Cribellata*.

I have followed Blackwall in considering the group as one family, and use his name *Ciniflonidæ*. The sub-family *Uloborinæ* of Thorell

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J. Blackwall. On the number and structure of the mammulæ employed by spiders in the process of spinning. Trans. Linn. Soc. London, vol. xviii, 1839.

P. Bertkau. Cribellum and Calamistrum. Archiv für Naturgeschichte, 1882.

July, 1888.

I use in the same sense, transferring it from the *Epeiridæ* to this family.

Several of the spiders described by Hentz under the name of *Theridion* are probably *Dictyna*. Of these, *T. sublatum*, *morologum*, and *foliaceum* belong to this genus without much doubt, though I cannot identify them with any species here described.

Blackwall mentions among spiders from Canada, *Ergatis* (*Dictyna*) *diligens, var. annulipes*, Ann. and Mag. of Nat. Hist., 1871.

B. G. Wilder describes the webs and habits of *Hyptiotes cavatus* under the name *H. Americanus*, in Popular Science Monthly, 1875.

E. Keyserling has described in Transactions of the Zool. Botan. Gesellschaft of Vienna, 1881 to 1884, the following species: *Dictyna sedentaria*, Baltimore, Coll. of L. Koch. *D. volupis*, Museum, Cambridge, Mass. *D. volucripes*, Museum, Cambridge, Mass. *D. foliata*, Colorado, Vienna Museum. *D. vittata*, Washington, D. C., Warsaw Museum. *D. arundinaceoides*, Cañon City, Colorado, Coll. of G. Marx.

## Dictyna Sundevall.

The genus *Dictyna* is composed of small spiders that live in loose webs of various shapes on fences and on plants, especially on the ends of stalks and among the flowers of *Solidago*, *Spiræa*, and other slender plants with clusters of small flowers.

The head is generally high, but not so wide as in *Amaurobius*. The sternum is very wide and convex and the labium large, often nearly as long as the mandibles. The tracheæ are large and the opening generally distinct. The difference between the sexes in most species is very great. The male palpi are very large and the palpal organs conspicuous. The tibial joint of the male palpi has on the outer side a process with two short spines. The mandibles of the males are bowed outward (Plate IX, fig. 2d) and are much longer than those of the female. They are bent forward at the tips, and at the base of each mandible is a short tooth projecting forward. Plate IX, fig. 2*b*.

The colors are usually dull yellow and brown covered with white or gray hairs. The cephalothorax is usually lighter in front and dark at the side, and the abdomen has a double row of light markings in the middle on a dark ground, but these markings are extremely variable even in the same species. Pl. IX.

The webs of *Dictyna* usually radiate irregularly from a hole or hiding place where the spider hangs. Some species, living on walls,

make a round patch of web with the hole near the center, but usually the shape of the web depends on that of the plant on which it is made. The principal threads of the web, if they are parallel or radiating slightly, are often crossed by a number of parallel short threads, like a segment of a web of *Epeira* (Pl. XI, fig. 3) and the curled band is carried back and forth on these as in the figure of the web of *Amaurobius*. Pl. X, 1*g*.

**Dictyna muraria**, new sp.

PL. IX, FIGURES 1 TO 1*g*.

Length about 3$^{mm}$. The cephalothorax is dark brown, a little lighter on the top of the head with a few gray hairs in longitudinal rows. The abdomen resembles that of *D. volucripes* Keys, but the middle dark markings are wider in front and more broken behind. In the middle of the front half is a wide dark patch, extending about to the middle of the abdomen. Behind this are two rows of dark spots connected by transverse lines, more or less complete, forming an Epeira-like marking. Pl. IX, figs. 1 to 1*e*.

The markings of this species and of *volucripes* vary greatly, so that they often cannot be distinguished by them. The metatarsus of the hind legs is nearly straight, not so much curved as in *volucripes*.

The males are darker, but usually have the same markings. Their abdomen is smaller than that of the females, but the cephalothorax is fully as large. The male palpi resemble those of *volucripes* Keys. The tibia is similar in shape, but is proportionally longer, and the two-spined process shorter than in *volucripes*. Pl. IX, 1*f*, 1*g*.

This spider is found all over New England. It is the most common species on fences, but is found also on plants, and, in winter, under leaves. It sometimes tries to fly, oftener in the spring than in the fall, which is the usual flying time of most spiders. I have specimens from Mt. Washington, N. H.; Portland, Me.; Salem, Mass.; Albany, N. Y.; New Haven, Conn.; Wood's Holl, Mass.; and several places around Boston.

A female in the Museum of Zoölogy, Cambridge, Mass., named by Keyserling, *D. arundinaceoides* Keys., is perhaps this species. It has the abdomen very much distended, so that the epigynum shows much plainer than usual. The spider first described by Keyserling as *D. arundinaceoides* came from Colorado, and I have not seen it and do not feel sure enough of its identity to adopt the name for this species.

**Dictyna volucripes** Keyserling, Zoöl. Botan. Gesellschaft, Vienna, 1882.

PL. IX, FIGURES 2 TO 2*f* AND PL. XI, FIGURE 3.

Female, 3·5$^{mm}$ long or longer. The male is nearly as large, but the cephalothorax is larger and the abdomen smaller.

The cephalothorax is dark reddish brown, and partly covered with white or gray hairs, most of them arranged in several lines from the dorsal groove to the eyes.

The abdomen has an irregular dark figure in the middle, narrow in front and widening backward. On each side of this is a light gray area, which becomes yellow in alcohol, and below these the sides and under surface of the abdomen are dark brown with some light markings. The abdomen is covered with gray hairs which modify the color. The legs are brown, usually lighter than the thorax, and covered with gray hairs.

The male is a little darker than the female. The male palpi are short, and large at the end. The patella is as wide as long. The tibia is a little longer than the patella and widened on the outer side at the distal end, so as to be as wide there as long. The two-spined process is as long as the tibia is wide, and is on the upper side of the tibia. The tarsus and palpal organ are large. Pl. IX, figs. 2*e*, 2*f*. The two spined process varies in form. It is usually curved forward, but in some specimens is nearly straight.

This species lives most commonly in thick and irregular webs on the ends of plants. The dried tops of *Spiræa* and *Solidago* are favorite places for it. It also lives occasionally on fences. All over New England.

**Dictyna longispina**, new sp.

PL. IX, FIGURE 4.

This species resembles *volucripes*, but is a little smaller. The markings of the abdomen are similar, but the cephalothorax and legs are lighter and redder.

The plainest difference of this species from the others is in the shape of the tibia of the male palpus. This is very long, as long as the femur, and stouter. The two-spined process is as long as the tibia, and extends backward nearly parallel to it. Pl. IX, fig. 4. The palpal organ extends farther backward than usual. The end of the tube and the accompanying process extending in a spiral nearly to the base of the tibia.

A young female, similarly marked and colored, accompanies the male and is probably the same species.

Meriden, Conn., one male and one female.

**Dictyna bostoniensis**, new sp.

PL. IX, FIGURES 3 TO 3*d*.

This is a rather large species, measuring $4^{mm}$ or more in length, but the cephalothorax is small and the abdomen much larger than in most species. The legs are whitish. The cephalothorax of the female is the same color in the middle, but darker and streaked with radiating brown lines on the sides. The abdomen is white with gray or black markings. Pl. IX, figs. 3*b*, 3*c*, 3*d*. In the middle of the front half of the abdomen is an irregular dark stripe extending over the first and second segments. Behind this are two rows of irregular spots, about one-third the width of the abdomen apart. The sides are marked by a few dark spots in broken oblique lines. The sternum and under side of the abdomen are white, with a few irregular dark spots. The spider resembles a piece of bird dung.

The cephalothorax of the male is larger and darker colored. The male palpi are short and slender. The tibia is short and as wide at the distal end as it is long. The two-spined process is short and on the outer side. The tarsus and palpal organ are small. Pl. IX, fig. 3*a*.

In 1873, this spider lived in great numbers on the iron fence around the public garden in Boston, making webs in corners, with an open tube in which the spider stood. Single specimens were found in Beverly and Brookline. In 1886, it was rare on the public garden, but common on the fences of the Back Bay park on Beacon street. I have not found it in other parts of New England.

**Dictyna minuta**, new sp.

PL. IX, FIGURES 5, 5*a*.

About $2^{mm}$ long. The markings are like those of *D. muraria*, but the colors are lighter and redder than in that species, and the only two specimens are much smaller.

The legs are very light brownish yellow, darker toward the base. The sternum and labium are reddish brown, and both are large and wide in proportion to the size of the spider.

The tibia of the male palpus is about twice as long as the patella, and nearly straight. The two-spined process is short and turned slightly forward. The spines are large and black. The point of the palpal organ is long and slender and twisted loosely. In both specimens it reaches backward half the length of the tibia. Pl. IX, figs. 5, 5*a*.

Two specimens only, from Hamden, Conn., and Providence, R. I.

**Dictyna rubra**, new sp.

PL. IX, FIGURE 7.

Female 2·5$^{mm}$ long. The color is more red than in the other species. The cephalothorax and legs are light orange-brown. The abdomen is darker reddish brown, with several yellow cross lines on the hinder half, and in some individuals a yellow patch on the front half. The sternum, maxillæ, and mandibles are light, like the legs.

The male palpi are moderately large. The tibia is a little longer than wide, and as thick at the base as at the tip. The two-spined process is on the upper side of the tibia, close to the base. It is as long as the tibia is thick. The tarsus is small. Pl. IX, fig. 7.

The abdomen is more pointed behind than in most species.

It lives on plants, but I do not know its web. Common in eastern Massachusetts and around New Haven, Conn.

**Dictyna cruciata**, new sp.

PL. IX, FIGURES 6, 6*a*.

This is the lightest colored species. The cephalothorax of the female is yellowish white in the middle and light brown at the sides. In the male the whole cephalothorax is dull yellow.

The abdomen is white in the middle, the color spreading down the middle of each side, forming in many individuals a cross-shaped marking (Pl. IX, fig. 6). The sides are light brown. The legs are yellowish white.

The male palpi are large and the palpal organs wide. The tibia is short, not much longer than wide, and the two spines are short and on a low process of the tibia. Pl. IX, fig. 6*a*.

Eastern Mass.; New Haven, Conn.

**Dictyna volupis** Keyserling, Zoöl. Botan. Gesell., Vienna, 1882.

PL. IX, FIGURES 8 TO 8*c*.

This is one of our most common spiders throughout the summer. It lives under leaves and between the twigs of trees and shrubs of all kinds, making small and thin webs.

The female is about 3$^{mm}$ long. The legs and front part of the cephalothorax are yellowish white. The sides of the cephalothorax are brown. The abdomen has usually an irregular light yellow marking in the middle and is light brown or reddish at the sides. Pl. IX, fig. 8. Some individuals are without the yellowish marking on the back and have the abdomen brownish all over, covered with whitish hairs. The reddish markings all become redder in alcohol. The sternum and under side of the abdomen are light yellow.

The colors of the male are very different. The whole cephalothorax is orange-brown, not much darker at the sides. The abdomen is reddish brown, darker than in the female, with only a little yellow in the middle, and sometimes none. The legs are darker yellow than in the female.

The males and females are about the same size, but differ in form as much as they do in color. The front of the head is low in both sexes, and rises backward to its highest point midway between the eyes and the dorsal groove. In the males the mandibles are so long that the distance from the top of the head to the end of the mandibles is as great as the length of the cephalothorax. The male mandibles are concave in front and bowed widely apart in the middle. Even the females have the mandibles a little concave in front.

The male palpi are long and large. The tibia is twice as long as wide; thickened at the end, and curved downward. The two-spined process is short and a little in front of the base of the tibia. Pl. IX, fig. 8*c*.

The palpal organ is unusually large, and the long tube can be seen passing around it under the edges of a large thin appendage. Pl. IX, fig. 8*a*, 8*b*.

The webs are spread under leaves or between twigs.

I have twice seen the pairing of this species. In one case the female stood across a forked twig and the male reached up from below, his head being under hers and his mandibles parallel to her sternum. In the other the male and female stood head to head in the web, the cephalothorax of the female being tipped up in front, and resting upon the head and mandibles of the male.

Common all over New England. In winter they are often found under leaves, half grown, and soon get to their growth when warm weather begins. Several small, flat egg-cocoons are fastened under a leaf and there may be several broods in each season.

**Dictyna frondea**, new sp.

PL. IX, FIGURES 9, 9*a*.

This species is a little smaller than *volupis* and is similarly colored in the female, except that it is usually a little darker and less red. The cephalothorax is light brown, a little lighter in the middle of the head, but not so much so as *volupis*. The abdomen is brown at the sides and yellow in the middle. The yellow stripe is narrower and straighter than in *volupis*, and often forms a regular herringbone figure. Pl. IX, fig. 9. The under side of the abdomen

is nearly as dark as the upper side, and the sternum has the same color while *volupis* is usually light colored beneath.

The males differ less from the females than in *volupis*. They are colored a little darker than the females. The light stripe on the abdomen is narrower and in some individuals wanting. The male palpi are long and slender. The tibia is more than twice as long as wide. The two spined process is very small and close to the base of the tibia. The tarsus is smaller than in *volupis*, and the palpal organ very much smaller and more simple. Pl. IX, fig. 9*a*.

On grass and low bushes all over New England.

This species (or *D. volupis*) is probably Hertz's *Theridion foliaceum*.

### Amaurobius C. Koch.

The genus *Amaurobius* contains our largest spiders of this family. In general appearance they resemble the stouter species of the genus *Tegenaria*, but do not have the long upper spinnerets of that genus. The head is large and high, and wide in front. The eyes are in two rows, only slightly curved, and are all small and of nearly the same size.

The maxillæ are long, and at the tip curve inward a little over the labium. The labium is longer than wide and a little narrowed at the tip. The mandibles are very large and strong.

The whole body is thickly covered with fine, short hair, giving it a soft velvet-like appearance. The spines on the legs are small and concealed by the hair.

The calamistrum consists of two rows of hairs, those of the outer row being much curved and close together, and those of the inner row three or four times as far apart. Pl. x, fig. 1*f*. The cribellum is long and narrow and divided in the middle. Pl. x, fig. 1*e*.

The colors of all our species are much alike. The cephalothorax and legs are dark brown, except in freshly moulted or young specimens, and the abdomen is dark gray with a double row of oblique light markings.

The webs are large and loose, often filling a cavity in a rotton log or under stones. There appears to be little regularity in the shape of the web or arrangement of the threads. The whole web is made of smooth silk and the band of curled threads is afterwards attached to parts of it as in Pl. x, fig. 1*g*.

The sexes are about equal in size. The male palpi are large. Their tibial joints are short and wide and furnished with large processes of various shapes.

**Amaurobius sylvestris**, new sp.

PL. X, FIGURES 1 TO 1*g*.

This is the common *Amaurobius* all over New England. The female is $10^{mm}$ long with the cephalothorax $5^{mm}$ long. The head is nearly as wide as the thorax. It is low in front and rises to its highest point half way to the dorsal groove. The cephalothorax is dark brown, darkest on the front of the head. The legs are dark brown, usually lighter than the thorax. In the young the colors are all much paler.

The abdomen is oval, widest behind. It is dark greenish gray with a double row of oblique yellow or white markings on the hinder half, and two curved markings of the same color on the front. These markings run together, forming a figure which varies greatly in form and size in different individuals. Pl. x, fig. 1.

The males differ but little from the females. The male palpi are large. The tibia is short and wide and has three long processes, the inner of which is slender and pointed and nearly twice as long as the tibia (Pl. x, figs. 1*a*, 1*b*.) but not so much curved as in figures of the European *A. claustrarius*.

The epigynum is small, the middle lobe is small and the side lobes meet behind so as to completely surround it (Pl. x, fig. 1*c*) which is very different from the epigynum of *A. claustrarius* as figured by Koch.

This species lives under stones, under leaves, and in the hollows of rotton trees and stumps. Fig. 1*g* is part of a web, showing the arrangement of the curled threads.

All over New England. In the White Mountains up to the highest trees.

Three specimens of this species in the museum of Comp. Zoölogy, Cambridge, are named by Keyserling *A. claustrarius*, which this species closely resembles. I have only young *claustrarius* for comparison, but judging by descriptions and figures, especially those of L. Koch in Abh. Nat. Gesellsch. of Nuremburg, 1868, I do not believe them the same species.

**Amaurobius ferox** (Walck.) Koch., *Cinifio ferox* Blk.

PL. X, FIGURES 3 TO 3*c*.

This is our largest species. It is found only about houses and cellars, and is probably imported, as it is a common spider in Europe.

The female is $12^{mm}$ long. Cephalothorax $6^{mm}$ long, $4^{mm}$ wide. The head is $3^{mm}$ wide and highest half way between the eyes and the dorsal groove.

The cephalothorax is yellowish brown, darkest in front and nearly black around the eyes. The legs are the same color as the thorax, darkest toward the tips. The abdomen is dark gray with light yellowish marks on the back. On the front half of the abdomen are a middle and two lateral stripes and behind these four or five pairs of oblique markings.

The eyes are all small and about equal in size. The front row is about half the width of the head and the eyes equidistant. The upper row is longer and the lateral eyes considerably farther from the middle ones than these are from each other. The mandibles are large and strong.

The calamistrum is a double row of spines, half the length of the hind metatarsus.

The male differs but little from the female, except that the abdomen is a little smaller and the front legs longer. The male palpi are very large. The tibia is as short as wide. It is bent inward, and has a large spine on the outer and another on the upper side, each nearly as long as the tibia. On the inner side is a third smaller spine. Pl. x, figs. 3*a*, 3*b*, 3*c*. The tarsus and palpal organ are large and round. Fig. 3*a*. The epigynum is large and dark colored. The middle lobe is large and enclosed by the others only at the sides. Pl. x, fig. 3.

Boston, Salem, Beverly, Mass.; Providence, R. I.; Albany, N. Y.; New Haven, Conn., in cellars and houses.

**Amaurobius tibialis**, new sp.

PL. X, FIGURES 3 TO 3*c*.

Female $8^{mm}$ long.

The cephalothorax is light brownish yellow, not darker in front. The legs are of the same color and not much darker at the tips.

The light markings on the abdomen are united into a middle band with oblique branches at the sides on the hinder half.

The middle lobe of the epigynum is entirely concealed, the lateral lobes divided by a groove in the middle. Pl. x, fig. 2.

The middle process on the tibia of the male palpus is short, but the other processes are much larger than in the other species. The outer one is about as long as the tibia is wide, and has a large hook on the inner side. The inner process is long and slender, curving over the back of the tarsus and extending nearly to the end of it. Pl. x, figs. 2*a*, 2*b*.

This species is found on Mt. Washington, N H., up to the highest trees.

## Titanœca Thorell.

### Titanœca americana, new sp.

PL. X, FIGURES 4 TO 4*d*.

This spider resembles *T. quadriguttata* of Europe, but is usually without markings on the abdomen. The female is 5 or 6$^{mm}$ long, resembling in size and shape the common *Steatoda borealis*, from which, however, it is readily distinguished by its black color.

The cephalothorax is dull orange-color, blackish around the edges and toward the front. The rest of the body is deep black and covered with long hair, except in some individuals a few light gray spots in pairs on the abdomen.

The sternum is as wide between the second legs as it is long. The labium is as wide as long, a little narrowed and rounded at the tip. The maxillæ are nearly straight on the inner edges not curved inward at the tips, as in *Amaurobius*. The head is not so wide as in *Amaurobius*. The eyes have nearly the same arrangement, but are proportionally larger. Pl. x, fig. 1. The spinnerets are short.

The cribellum is divided in the middle as in *Amaurobius*, and the calamistrum is like that genus.

The claws of the feet are large and strong, proportionally larger than those of *Amaurobius*.

Like most of the genus, this lives under stones in the driest and hottest places. Under the loose stones under the trap hills around New Haven and Meriden, Conn. it is common. I have a few from Mt. Monadnock, N. H., but have not found it elsewhere in New England.

### Titanœca brunnea, new sp.

PL. X, FIGURES 5 TO 5*c*.

This species is about as large as *T. americana*, but is a little more slender and less hairy. The cephalothorax is light or dark brown, like dead oak leaves among which it lives. The joints of the legs are darker toward the distal ends. The abdomen is similarly colored, but becomes redder than the rest of the body in alcohol. Across the back are four or five lines of light yellowish spots, and there are larger irregular spots along the sides, as in many species of *Dictyna*. Pl. x, figs. 5, 5*a*. Under the abdomen between the spinnerets and epigynum are two large light spots. Fig. 5*a*.

Besides the color, the only plain difference between this and the black species is in the palpi of the female, which in this species have

the last two joints a little stouter than in *T. americana*. In both species the palpi are very spiny at the end, though the spines are concealed by hairs. I have found this species three times under leaves in woods near New Haven, Conn.

## Uloborinæ Thorell.

These spiders have been classed by most authors among the *Epeiridæ* on account of their resemblance to *Tetragnatha*, and especially on account of their round, or at least radiate, webs. The arrangement of the eyes, the mouth parts, and the tracheæ are all different from the *Epeiridæ*. The spinning organs include the cribellum and calamistrum, like the other *Ciniflonidæ*, and the cross-threads of the webs are partly made of curly threads spun by the calamistrum and not covered with a liquid in drops like the webs of *Epeiridæ*.

The adhesive thread of these spiders is not made separately and attached to old threads as it is by *Amaurobius* and *Dictyna*, but both threads are spun at the same time. Pl. XI, figs. 2*i*, 2*j* show both sides of a piece of the cross-threads of the web of *Hyptiotes*.

### Uloborus Latreille. *Veleda*, Blackwall. *Phillyra*, Hentz.

### Uloborus plumipes Lucas = *Phillyra ripaira*, Hentz.

PL. XI, FIGURES 1 TO 1*f*.

The female is about 5$^{mm}$ long. Cephalothorax 1·5$^{mm}$. The cephalothorax is flat in front, and extends forward in the middle beyond the mandibles. Behind it is wide and swelled up on each side, where the abdomen hangs over it. Pl. XI, fig. 1*b*.

The abdomen is narrow and slightly notched in front and extends over the cephalothorax a quarter of its length. The abdomen is widest and highest a third of its length from the front, and at this point has a pair of humps.

The colors are very variable. A dark, plainly marked female has the femur and patella of the front legs dark brown or nearly black, and the tibia dark brown, except a white ring at the base; at the end of the tibia is a brush of coarse, dark brown hairs. The tarsus and metatarsus are white. In lighter individuals the color of the femur or tibia may be broken by a white ring near the middle. The other legs have femur, tibia and metatarsus dark brown, divided near the middle by a white ring. Patella and tarsus brown, lighter at the ends. The cephalothorax is dark brown with a narrow, indis-

tinct light line in the middle. In lighter individuals this stripe is wider.

The dorsal markings of the abdomen are more variable and less definite. On the front of the abdomen are two light spots, behind which are two very dark ones, sometimes united into one. Behind these are two white spots half as far apart as the humps and a little in front of them. The humps are generally dark on the inner side and light on the outer. Farther backward are two or three pairs of light spots, surrounded by a darker brown area, darkest in the middle and toward the spinnerets.

The sternum is brown, and the under side of the abdomen is dark in the middle and light at the sides. In lighter individuals most of these markings can be seen, the darker ones being light brown or yellow, and the lighter ones yellow or dirty white. In some no markings can be defined.

The first pair of legs is twice as long as the second, and much longer than the fourth pair.

The terminal joint of the palpus is more than twice as long as the one before it. The palpal claw is large, with two or three teeth. Pl. XI, fig. 1*b*.

The eyes are in two rows. The upper are largest and appear still larger on account of being surrounded by dark rings. Pl. XI, figs. 1*a*, 1*b*. They are on the top of the head, the lateral pair farthest back. The front row of eyes is close to the edge of the head, just over the mandibles.

The mandibles are small and rounded forward at the base. The maxillæ are as wide as long, with the front ends nearly square. The labium is triangular. (See figure of same parts in *Hyptiotes*, Pl. XI, fig. 2*h*.)

The male is much smaller than the female. The cephalothorax is more pointed in front and lower behind. The abdomen is not much larger than the cephalothorax and not so plainly humped as in the female. The legs are but little shorter than those of the female and the markings and colors are the same. The first tibia does not have a bunch of hairs at the end like the female. The palpal organ is nearly spherical, all the parts being wound closely together. Pl. XI, figs. 1*d*, 1*c*. The femur of the male palpus has, at the base, a short process on the outer side. Pl. XI, fig. 1*f*.

The webs are round and nearly horizontal, the cross-threads usually less regular than in webs of *Epeira*. The webs are commonly made between loose stones, but sometimes in low bushes. The cocoons are

half an inch long and quarter as wide, narrowed at both ends, and with numerous short points by which they are attached to the web around them. I have found them with the female under stones. The cocoons are light brown, and each female appears to make several of them. The cocoons are made in July.

This spider is found all over New England, but is not common anywhere. I have taken them from several places around Boston, Mass., in New Haven, Conn., and in Simsbury, Conn.

I have specimens of both sexes from the southern part of France, given me by Mr. E. Simon. It is found in Italy and Spain. The common *Uloborus* of the north of Europe (*U. walckenœrius*) is a very different species.

## Hyptiotes Wlk. = *Mithras*, Koch.

### Hyptiotes cavatus.

Pl. XI, figures 2 to 2*k*.

This peculiar spider is without much doubt the one described and figured by Hentz under the name of *Cyllopodia cavata*, although he saw but six eyes and four spinnerets, and otherwise described it incorrectly.

Its habits have been well described by B. G. Wilder in the Popular Science Monthly, 1875, where he calls it *Hyptiotes americanus*.

This spider resembles a shortened *Uloborus*. The adult female is about $4^{mm}$ long, and is colored like the end of one of the dead pine branches among which it usually lives.

The cephalothorax is as broad as long, highest in the middle just behind the eyes, and hollowed behind under the front of the abdomen.

The abdomen is oval, a little flattened in front. On the back are four pairs of low humps, the second largest, on each of which are a few stiff hairs.

The arrangement of the eyes resembles that in *Uloborus*, but the eyes are farther apart and farther back on the thorax. Pl. xi, figs. 2, 2*a*.

The legs are short and thickest in the middle, tapering distinctly from the patella to the claws. The hind metatarsus bearing the calamistrum is curved inward on the outer side. The claws have three or four teeth and under the claws are a few curved spines, some of which are toothed as in *Epeira*. Pl. xi, fig. 2*e*, *f*, *g*. The palpal claw has four or five teeth.

The mandibles are very small and slightly arched forward near the base.

The maxillæ and libium are like those of *Uloborus.* (Pl. XI, fif. 2*h*.)

The spinnerets are long. The cribellum is small and not divided in the middle.

The male is about half as large as the female. The abdomen is much smaller and the humps lower. The palpal organ is very large. The tube is long and slender and extends one and a half times around the organ, supported by the edge of a thin appendage. At the tip it lies against two small flexible processes and over them is a large dark horn. The whole apparatus is so large as to cover the patella as well as the tarsus. Pl. XI, figs. 2*c*, 2*d*.

The epigynum is simple externally, but the inner tubes correspond in length to those of the palpal organs.

The colors of both sexes are various shades of brown, covered with white or gray hairs. The markings on the cephalothorax and legs are usually indistinct. The eyes are surrounded by black rings. The humps on the abdomen are usually darker than the rest of the back. Dark markings follow the dorsal vessel and two or three pairs of its branches. Other individuals have the front, and some the whole back of the abdomen very dark brown.

The web consists of four rays crossed by a dozen or more threads. The point where the rays meet is attached to a thread which extends to the spider's roost, usually the end of a twig. Here it holds by the hind feet and draws the thread tight with the fore feet. When an insect strikes the web the spider lets go with the hind feet, the elasticity of the web draws the thread out with a snap, and slides the spider along it toward the web. When it reaches the center it feels the rays to find where the insect is, runs out on the nearest one, covers the prey with silk, and carries it out of the web.

The making of this web is fully described by Wilder. Having finished the rays, the spider begins with the cross threads farthest from the center, walking along the upper ray until it is near enough the next to step across, then it crosses and walks outward again on the second ray. The new cross thread is elastic enough to shorten to the proper length when she reaches the point to attach it. When the cross thread is finished to the fourth ray, the spider walks back to the center and out on the upper ray to the point for beginning another.

This spider is common all over New England and the Middle States. I have seen cocoons near their webs, like that described by Wilder, but have never traced it to them or any other spider.

## EXPLANATION OF PLATES.

### Plate IX. *Dictyna.*

Figs. 1, 1*a*, 1*b*, 1*c*, 1*d*, 1*e*. Dorsal markings of different individuals of *Dictyna muraria.*

Figs. 1*f*, 1*g*, Palpi of male *D. muraria.*

Fig. 2, *D. volucripes*, sternum and mouth parts: 2*a*, side of female; 2*b*, side of male; 2*c*, front of head and mandible of female; 2*d*, front of head and mandible of male; 2*e*, 2*f*, palpal organ and male tibia of different individuals.

Fig. 3, 3*a*, palpus of male *D. bostoniensis*; 3*b*, 3*c*, 3*d*, dorsal markings of *D. bostoniensis.*

Fig. 4, Tibia of male palpus of *D. longispina.*

Fig. 5, 5*a*, Male palpus of *D. minuta.*

Fig. 6, Common dorsal marking of *D. cruciata;* 6*a*, male palpus of *D. cruciata.*

Fig. 7, Palpus of male *D. rubra.*

Fig. 8, Dorsal markings of *D. volupis;* 8*a*, 8*b*, male palpus of *D. volupis;* 8*c*, tibia of male palpus seen from below.

Fig. 9, Dorsal markings of abdomen of *D. frondea;* 9*a*, palpus of male of *D. frondea.*

### Plate X. *Amaurobius.*

Fig. 1, *Amaurobius sylvestris*, × 4; 1*a*, tibia and patella of right palpus of male: 1*b*, tarsus and palpal organ; 1*c*, epigynum; 1*d*, foot; 1*e*, cribellum: 1*f*, calamistrum; 1*g*, part of web showing the arrangement of the curled threads.

Fig. 2, *Amaurobius tibialis*, epigynum: 2*a*, upper side of right palpus of male: 2*b*, outer side of the same.

Fig. 3, *Amaurobius ferox*, epigynum; 3*a*, palpal organ of male; 3*b*, tibia of male palpus, upper side; 3*c*, the same, outer side.

Fig. 4, Head of *Titanœca americana;* 4*a*, 4*b*, palpus of male; 4*c*, palpus of female: 4*d*, cribellum.

Fig. 5, Dorsal markings of *Titanœca brunnea;* 5*a*, under side of abdomen of the same; 5*b*, sternum, maxillæ and labium; 5*c*, palpus of female.

### Plate XI.

Fig. 1, *Uloborus plumipes*, side of female; 1*a*, top of cephalothorax and eyes 1*b*, side of cephalothorax and mouth parts; 1*c*, male; 1*d*, 1*e*, palpus of male.

Fig. 2, *Hyptiotes cavatus*, × 8, female: 2*a*, the same, male; 2*b*, calamistrum: 2*c*, palpus of male; 2*d*, palpal organ from below; the place of the large terminal process is shown by a dotted line; 2*e*, *f*, *g*, first and second feet; 2*h*, labium and maxillæ; 2*i*, 2*j*, thread of web of *Hyptiotus cavatus*, showing opposite sides; 2*k*, Diagram of web.

Fig. 3, Web of *Dictyna volucripes.*

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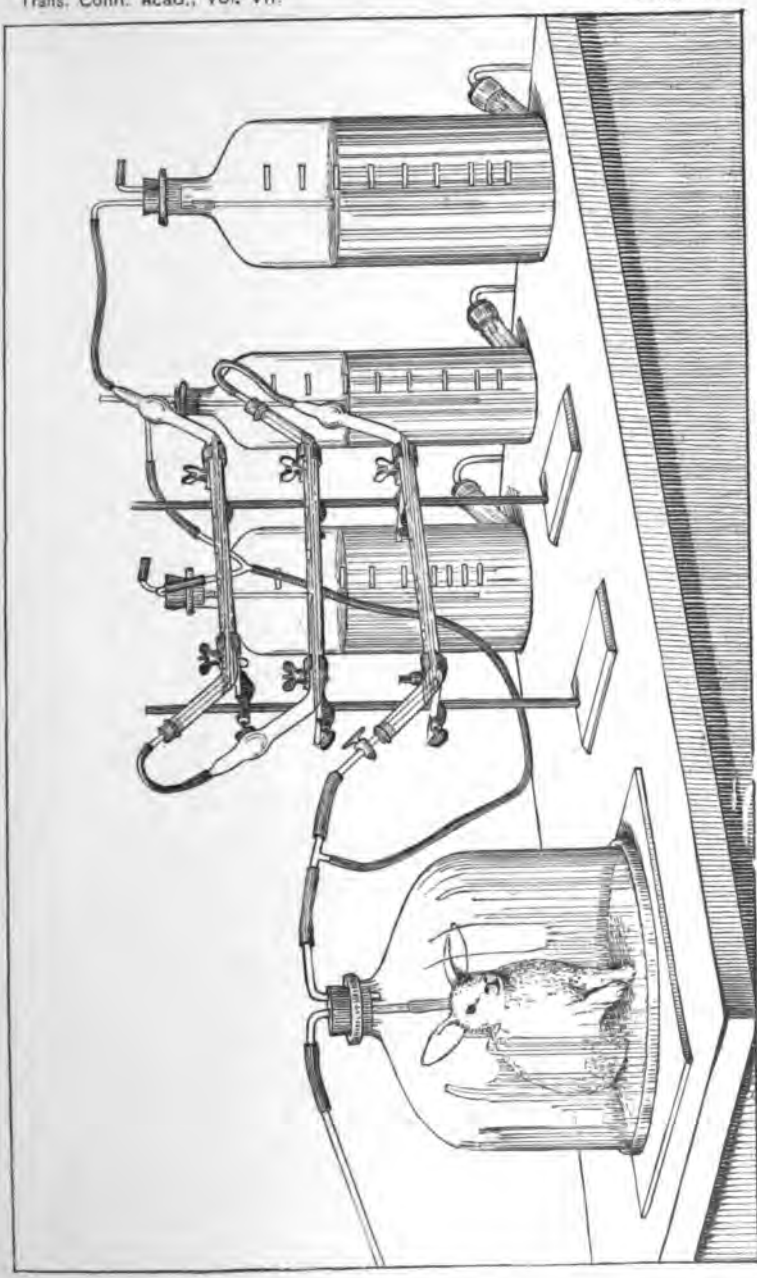

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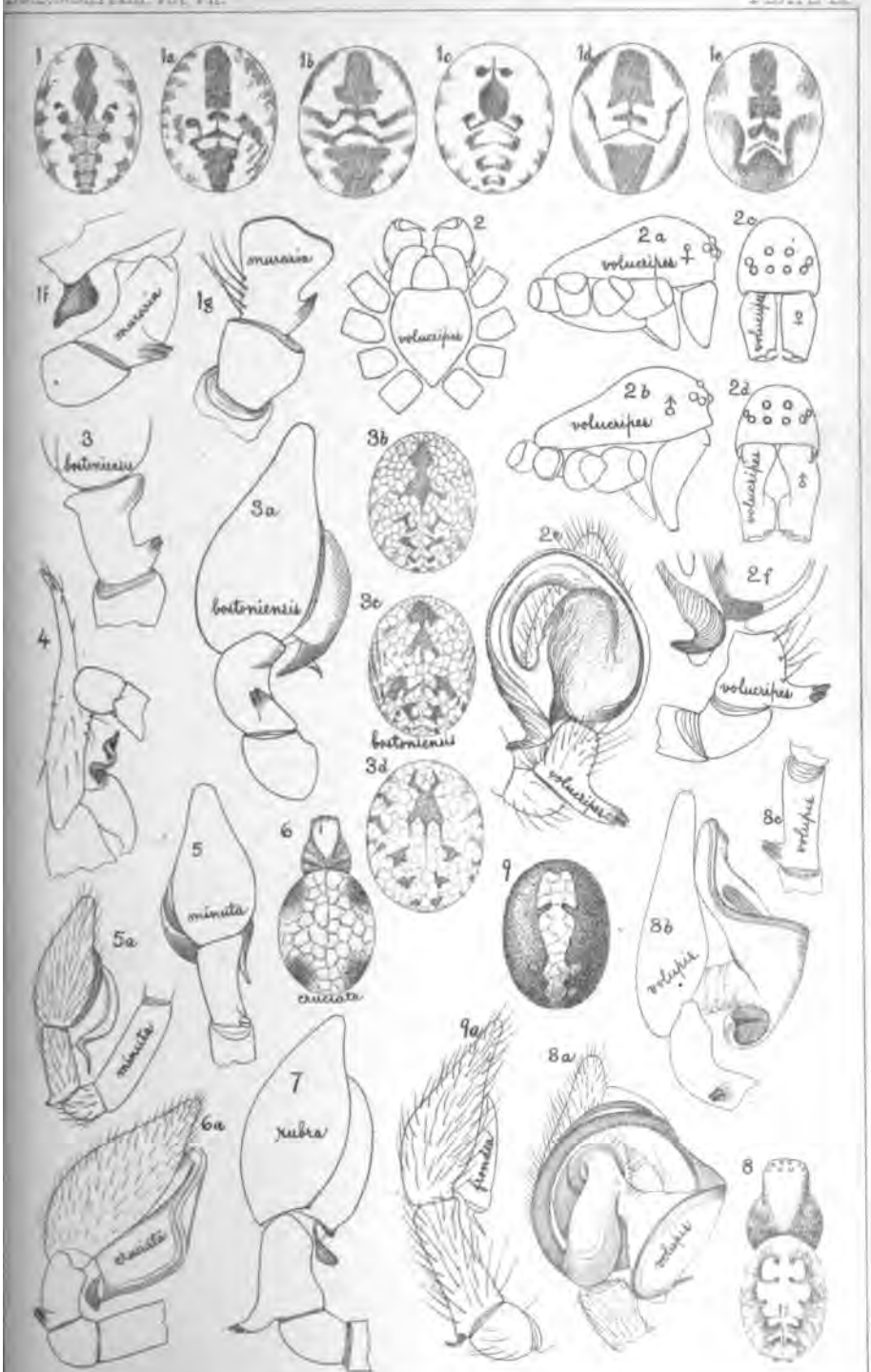
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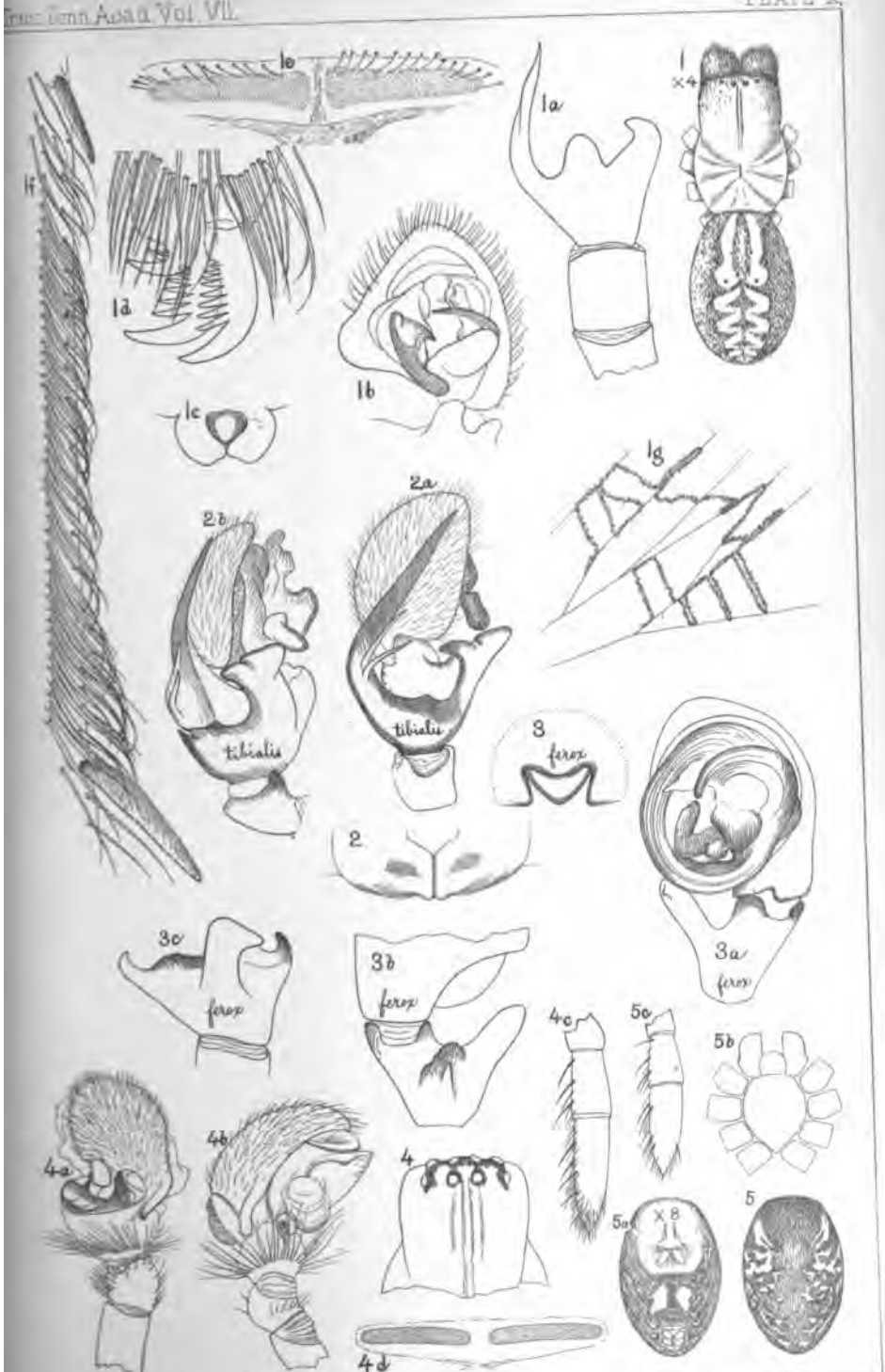


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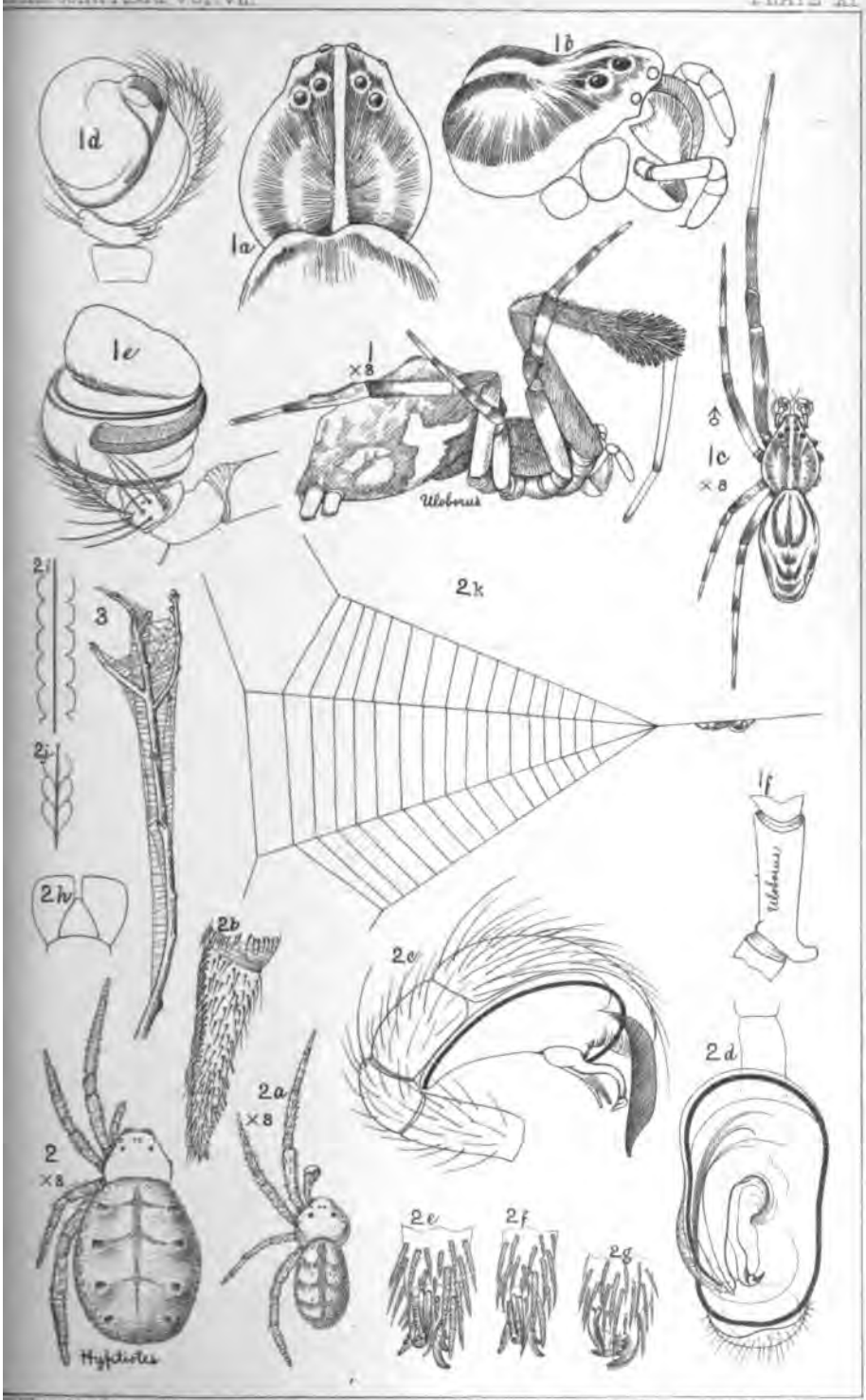


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Bullettino mensile. Nuova serie. Fasc. 1, 4-13, 1888-90. 8°.

CHERBOURG.—*Société Nationale des Sciences Naturelles.*

Mémoires. Tome XXVI, 1889. 8°.

CHRISTIANIA.—*Kön. Universitäts-Sternwarte.*

Zonenbeobachtungen der Sterne zwischen 64° 50′ und 70° 10′ nördlicher Declination. 1888. 4°.

———*Norwegisches meteorologisches Institut.*

Jahrbuch. 1886, 1887. 4°.

———*Norwegian North-Atlantic Expedition*, 1876-78.

Publication XIX. 4°.

———*Videnskabs Selskabet.*

Forhandlingar. 1888, 1889. 8°.

CHUR.—*Naturforschende Gesellschaft Graubündens.*

Jahres-Bericht. Neue Folge. Jahrg. XXXI-XXXIII, 1886-89. 8°.

CORDOBA.—*Academia Nacional de Ciencias.*

Actas. Tomo VI and atlas, 1889. 4°.

Boletin. Tomo X. 2, 3, XI. 1-3, 1887-89 8°.

DANZIG.—*Naturforschende Gesellschaft.*

Schriften. Neue Folge. Bd. VII. 1, 2, 1888-89. 8°.

DIJON.—*Académie des Sciences, Arts et Belles-Lettres.*

Mémoires. 3e sér. Tome X. 4 sér. Tome I. 1887-89. 8°.

DORPAT.—*Gelehrte Estnische Gesellschaft.*

Sitzungsberichte. 1888-89. 8°.

———*Naturforscher-Gesellschaft bei der Universität Dorpat.*

Archiv für die Naturkunde Liv-Ehst-und Kurlands. Ser. I. Bd. IX. 5, 1889. 8°.

Sitzungsberichte. Bd. VIII. 3, IX. 1, 1888-89. 8°.

Schriften. V, 1890. 8°.

DRESDEN.—*Naturwissenschaftliche Gesellschaft Isis.*

Sitzungsberichte und Abhandlungen. 1888, 1889, 1890. 8°.

———*Verein für Erdkunde.*

Mittheilungen. 1888. 8°.

Festschrift zur Jubelfeier des 25jährigen Bestehens. 1888. 8°.

Jubileumschrift. Litteratur des Landes-und Volkskunde des Königreich Sachsen. Bearb. von Paul Emil Richter. 1889. 8°.

DUBLIN.—*Royal Irish Academy.*

Transactions. Vol. XXIX. 1-13. 1887-90. 8°.

Proceedings. Ser. II. Polite lit. and antiq. Vol. II. 8. Science. Vol. IV. 6. Ser. III. Vol. I. 1-3. 1888-90. 8°.

Cunningham memoirs. No. V, 1890. 4°.

Todd lecture series. Vol. I. 1, II, 1887-89. 8°.

DUBLIN.—*Royal Irish Academy.*
Irish manuscript series. Vol. II. 1, 1890. 8°.
List of papers published in the transactions, Cunningham memoirs, and Irish manuscript series, 1786–1886. 4°.
——*Royal Geological Society of Ireland.*
Journal. Vol. XVII. 2, 1885–87. 8°.
EDINBURGH.—*Botanical Society.*
Transactions and proceedings. Vol. I. 1–3, III. 2, IV, V, X, XI, XII. 3, XIII. 2, 3, XIV–XVII, 1841–1889. 8°.
Annual report and proceedings. I–VIII, 1836–44, 1855. 8°.
——*Geological Society.*
Transactions. Vol. V. 4, VI. 1, 1888–90.
——*Royal Physical Society.*
Proceedings. Vol. IX. 2, 3, X. 1, 1886–89. 8°.
——*Royal Society.*
Proceedings. Vol. XV, XVI, 1887–89. 4°.
EMDEN.—*Naturforschende Gesellschaft.*
Jahresbericht. LXXII–LXXIV, 1886–89. 8°.
ERFURT.—*Kön. Akademie gemeinnütziger Wissenschaften.*
Jahrbücher. Neue Folge. Heft XVI, 1890. 8°.
FIRENZE.—*Biblioteca Nazionale Centrale.*
Bollettino delle pubblicazioni Italiane ricevute per diritto di stampa. No. 62–119, 1888–90. 8°.
——*R. Istituto di Studi Superiori Practici e di Perfezionamento.*
Pubblicazioni. Sezione di filosofia e filologia.
Le seconde nozze del conjuge superstite. Studio storico di Alberto Del Vecchio. 1885. 8°.
I piu antichi frammenti del costituto Fiorentino raccolti e pubblicati da Guiseppe Rondoni. 1882. 8°.
——Sezione di Scienze fisiche e naturali.
Osservazioni continue della elettricità atmosferica istituite a Firenze dal Prof. Antonio Roiti. 1884. 8°.
Linee generali della fisiologia del cervelletto. Prima memoria del Prof. Luigi Luciani. 1884. 8°.
——Sezione di medicina e chirurgia.
Archivio della scuola d'anatomia patologica. Vol. II, 1882. 8°.
Esegesi medico legale sul methodus testificandi di Giovan Battista Codronchi. Pel Prof. Angiolo Filippi. 1883. 8°.
FRANKFURT A. M.—*Deutsche malakozoologische Gesellschaft.*
Nachrichtsblatt. Jahrg. XX. 7–12, XXI, XXII, 1888–90. 8°.
——*Senckenbergische naturforschende Gesellschaft.*
Abhandlungen. Bd. XV. 3, XVI. 1, 1888–90. 4°.
Bericht. 1888–90. 8°.
FREIBURG IN B.—*Naturforschende Gesellschaft.*
Berichte. Bd. II–IV, 1887–89. 8°.
GENÈVE.—*Institut National Genevois.*
Bulletin. Tome XXIX, 1890. 8°.
Mémoires. Tome XVII, 1886–89. 4°.
——*Société de Physique et d'Histoire Naturelle.*
Mémoires. Tome XXX, 1888–90. 4°.
——*Museo Civico di Storia Naturale.*
Annali. Ser. II. Vol. III–VI, 1886–89. 8°.
GIESSEN.—*Oberhessische Gesellschaft für Natur- und Heilkunde.*
Bericht. XXVI, XXVII, 1889–90. 8°.
GLASGOW.—*Geological Society.*
Transactions. Vol. VIII. 2, 1886–88. 8°.

GLASGOW.—*Natural History Society.*
Proceedings and transactions. New series. Vol. II, III. 1, 1886–89. 8°.
———*Philosophical Society.*
Proceedings. Vol. XIX, XX, 1888–89. 8°.
GÖTTINGEN.—*Königl. Gesellschaft der Wissenschaften.*
Nachrichten. 1888, 1889. 8°.
GÜSTROW.—*Verein der Freunde der Naturgeschichte in Mecklenburg.*
Archiv. Jahrg. XLII, XLIII, 1888–89. 8°.
HABANA.—*Real Colegio de Belen.*
Observaciones magneticas y meteorologicas. 1886. iv, 1887, 1888. i 4°.
HALIFAX.—*Nova Scotian Institute of Natural Science.*
Proceedings and transactions. Vol. I. 4, V. 3, 4, VI. 2–4, VII. 1–3, 1865–89. 8°.
——— *Department of Mines.*
Report. 1888, 1889. 8°.
HALLE.—*Kais. Leopoldinisch-Carolinische deutsche Akademie der Naturforscher.*
Leopoldina. Heft XXIV, XXV, 1888–89. 4°.
———*Naturforschende Gesellschaft.*
Abhandlungen. Bd. XVII. 1, 2, 1888. 4°.
Bericht. 1887. 8°.
———*Naturwissenschaftlicher Verein für Sachsen und Thüringen.*
Zeitschrift für Naturwissenschaften. Bd. LXI, LXII, LXIII. 1–3, 1889–90. 8°.
HAMBURG.—*Deutsche Seewarte.*
Archiv. Jahrg. X–XII, 1887–89. 4°.
Monatliche Uebersicht der Witterung. 1888 März-Dec., 1889, 1890 Jan.-Mai. 8°.
Deutsches meteorologisches Jahrbuch. 1887, 1888. 4°.
Ergebnisse der meteorologischen Beobachtungen, 1876–1885. 4°.
———*Naturwissenschaftlicher Verein.*
Abhandlungen. Bd. XI. 1, 1889. 4°.
——— *Wissenschaftliche Anstalten.*
Jahrbuch. Jahrg. VI, 1888.
HANNOVER.—*Naturhistorische Gesellschaft.*
Jahresbericht. XXXVIII, XXXIX, 1887–89. 8°.
HARLEM.—*Musée Teyler.*
Archives. Série II. Vol. II. 1, III. 2–4, 1884–90. 8°.
Catalogue de la bibliothèque. Vol. I. 7, 8, II. 1, 3. 1887–89. 8°.
———*Société Hollandaise des Sciences.*
Archives néerlandaises des sciences exactes et naturelles. Tome XXIII, XXIV, 1888–90. 8°.
HELSINGFORS.—*Societas Scientiarum Fennica.*
Acta. Tom. XV, XVI, 1888. 4°.
Öfversigt af förhandlingar. XXVIII–XXXI, 1885–89. 8°.
Bidrag till kännedom af Finlands natur och folk. Häft. XLV–XLVIII, 1887–89. 8°.
Finska Vetenskaps-Societeten, 1838–1888, dess organisation och verksamhet. Af. A. E. Arppe. 1888. 8°.
———*Societas pro Fauna et Flora Fennica.*
Acta. Vol. III, IV, V. 1, 1886–88. 8°.
Meddelanden. Häft. XIV, XV, 1888–89.
Herbarium musei fennici. Ed. 2. I. Plantae vasculares. 1889. 8°.
Notae conspectus florae fennicae. Auctore H. Hjelt. 1888. 8°.
HERMANNSTADT.—*Siebenbürgischer Verein für Naturwissenschaften.*
Verhandlungen und Mittheilungen. Jahrg. XXVIII. XXIX, 1888–89. 8°.

HOBART.—*Royal Society of Tasmania.*
Papers and proceedings. 1887, 1888. 8°.
JENA.—*Medicinisch-naturwissenschaftliche Gesellschaft.*
Jenaische Zeitschrift für Naturwissenschaft. Bd. XXII. 3, 4, XXIII, XXIV, 1888–90. 8°.
KHARKOW.—*Société des Sciences Experimentales annexée à l'Université de Kharkow.*
Travaux de la section médicale. 1886–87, 1888. i, 1889. i–iii. 8°.
KIEL.—*Königl. Christian Albrechts-Universität.*
Schriften aus dem Jahre 1887–88, 1888–89, 1889–90. 8° and 4°.
——*Naturwissenschaftlicher Verein für Schleswig-Holstein.*
Schriften. Bd. VII. 2, VIII. 1, 1889. 8°.
KIEV.—*Kievskie Obshchestvo Iestestvoispytateleï.*
Zapiski. Tom. IX. 1, 2, X. 1–3, XI. 1, 1888–88. 8°.
KJÖBENHAVN.—*Kon. Danske Videnskabernes Selskab.*
Oversigt over forhandlinger. 1887. iii, 1888, 1889, 1890. i. 8°.
KÖNIGSBERG.—*Königl. physikalisch-ökonomische Gesellschaft.*
Schriften. Jahrg. XXVIII–XXX, 1887–89. 4°.
KRAKÓW.—*K. k. Sternwarte.*
Materyaly do klimatografii Galicyi. Rok 1887, 1888. 8°.
LA PLATA.—*Museo.*
La musée de La Plata. Rapide coup d'œil sur sa fondation et son développement. Par F. P. Moreno. 1890. 8°.
LAUSANNE.—*Société Vaudoise des Sciences Naturelles.*
Bulletin. 3e sér. No. 97–101, 1888–90. 8°.
LEEDS.—*Yorkshire Geological and Polytechnic Society.*
Proceedings. New series. Vol. X, XI. 1-2, 1888–89. 8°.
LEIDEN.—*Nederlandsche Dierkundige Vereeniging.*
Tijdscrift. Ser. II. Deel II. 3, 4, supplement deel II, 1888–89. 8°.
LEIPZIG.—*Astronomische Gesellschaft.*
Vierteljahrsschrift. Jahrg. XXIII, XXIV, XXV. 1, 2, 1888–90. 8°.
Publication. XIX, 1889 4°.
Catalog. Abth. I. Stück IV, XIV, 1890. 4°.
——*Kön. sächsische Gesellschaft der Wissenschaften.*
Berichte. Math.-physische Classe. Bd. XL, XLI, XLII. 1, 1888–90.
Register zu den Jahrg. 1866–85 der Berichte und Bd. I–XII der Abhandlungen. 1889. 8°.
——*Naturforschende Gesellschaft.*
Sitzungsberichte. Jahrg. XIII, XIV, 1886–87. 8°.
——*Verein für Erdkunde.*
Mittheilungen. 1887–89. 8°.
——Zoologischer Anzeiger. No. 283–350, 1888–90. 8°.
LIÉGE.—*Société Géologique de Belgique.*
Annales. Tome XIII. 2, XIV. 2, XV. 2. 3, XVI. 1, 1888–89. 8°.
——*Société Royale des Sciences.*
Mémoires. 2e sér. Tome XV, XVI, 1888–90. 8°.
LISBOA.—*Sociedade de Geographia.*
Boletin. Serie VII. 9–12, VIII, IX. 1–6, 1887–90. 8°.
Catalogos e indices. As publicaçoes. 1889. 8°.
Indices e catalogos. A biblioteca. I. 1890. 8°.
Historia do infante D. Duarte irmão de el-rei D. João IV. Por José Ramos-Coelho. Tomo I. 1889. 8°.
LIVERPOOL.—*Literary and Philosophical Society.*
Proceedings. No. XLI–XLIII, 1887–89. 8°.
LONDON.—*Geological Society.*
Quarterly journal. Vol. XLIV. 3, 4, XLV, XLVI, 1888–90. 8°.

LONDON.—*Linnean Society.*
Journal. Zoology. No. 118–123, 130–144, 1888–90. 8°.
—Botany. No. 150, 152–157, 159–174, 181–182, 1887–90. 8°.
Proceedings. 1887–88. 8°.
List. 1888–90. 8°.
———*Mathematical Society.*
Proceedings. No. 321–390, 1888–90. 8°.
———*Royal Meteorological Society.*
Quarterly journal. New series. No. 67–76, 1888–90. 8°.
List of fellows. 1889, 1890. 8°.
———*Royal Microscopical Society.*
Journal. 1888. iv–vi, 1889, 1890. i–v. 8°.
———*Royal Society.*
Philosophical transactions. Vol. CLXXVIII, CLXXIX. A, B, CLXXI. A, B. 1887–89. 4°.
Proceedings. No. 270–294, 1888–90. 8°.
List of council and members. 1887–89. 4°.
LOUVAIN.—La Cellule. Tome I–V, VI. 1, 1884–90. 8°.
LUND.—*Universitet.*
Acta. Tom. VIII, XVIII, XXIV, XXV, 1871–89. 4°.
LYON.—*Académie des Sciences, Belles-Lettres et Arts.*
Mémoires. Classe des sciences. Tome XXVIII, XXIX, 1886–88. 8°.
Histoire des herbiers. Par le Dr. Saint-Lager. Paris, 1885. 8°.
Recherches sur les anciens herbaria. Par le Dr. Saint-Lager. Paris, 1886. 8°.
Vicissitudes onomastiques de la globulaire vulgaire. Paris, 1889. 8°.
———*Musée Guimet.*
Annales. Tome XIII, XV–XVII, 1888–89. 4°.
Revue de l'histoire des religions. Tome XVI, 2, 3, XVII–XXI, 1887–90. 8°.
MADRAS.—*Government Observatory.*
Observations made with the meridian circle, 1865–67. 4°.
MADRID.—*Comision del Mapa Geologico de España.*
Boletin. Tomo XIV, XV, 1887–88. 8°.
Memorias. Descripcion fisica, geologica y minera de la provincia de Huelva. Por D. Joaquin Gonzalo y Tarin. Tome I, II, 1886–88. 8°.
Mapa geologico de España. Hoja 6, 8, 12, 16, 19, 20, 23, 24, 27, 28, 31, 32. 1889.
———*Observatorio.*
Observaciones meteorologicas. 1886–87. 8°.
Resumen da las observaciones efectuadas en la peninsula. 1884, 1885. 8°.
———*Sociedad Española de Historia Natural.*
Anales. Tomo XVII. 2, 3, XVIII, 1888–89. 8°.
———*Real Academia de Ciencias Exactas, Fisicas y Naturales.*
Memorias. Tomo XIII. 2, 3, 1889. 4°.
Revista de los progresos de las ciencias exactas. Tomo XXII. 5–7, 1888–89. 8°.
Anuario, 1889. 8°.
MANCHESTER.—*Literary and Philosophical Society.*
Memoirs and proceedings. Series IV. Vol. I–III, 1888–90. 8°.
MARBURG.—*Gesellschaft zur Beförderung der gesammten Naturwissenschaften.*
Sitzungsberichte. Jahrg. 1888, 1889. 8°.
MELBOURNE.—*National Museum.*
Prodromus of the zoology of Victoria. Decade XVI–XX. 1888–90. 8°.
METZ.—*Académie.*
Mémoires. 3e sér. Année XV, 1885–86. 8°.

MEXICO.—*Observatorio Meteorologico-Magnetico Central.*
Boletin mensuel. Tomo I. 6-12, II, 1888-89. 4°.
——*Sociedad Cientifica "Antonio Alzate."*
Memorias. Tomo II, III, IV. 1-2, 1888-90. 8°.
——*Sociedad de Geographia y Estadistica.*
Boletin. Epoca IV. Tomo I. 1-4, 6, 1888-89. 8°.
——*Sociedad Mexicana de Historia Natural.*
La naturaleza. Ser. II. Tomo I. 4-9. 1888-90. 4°.
MIDDELBURG.—*Zeeuwsch Genootschap der Wetenschappen.*
Zelandia illustrata. Vervolg. 1885. 8°.
Levensberichten van Zeeuwen. Afl. I, II, 1888-89. 8°.
De stadsrekeningen van Middelburg. III. 1500-1549. Door H. M. Kesteloo. 8°.
Vluchtbergen in Walcheren. Door Dr. J. C. Man. 1888. 8°.
MILANO.—*Real Istituto Lombardo di Scienze e Lettere.*
Rendiconto. Serie II. Vol. XX, XXI, 1887-88. 8°.
——*Real Osservatorio di Brera.*
Pubblicazioni. No. XXXIII-XXXVII, 1888-91. 4°.
——*Società Italiana di Scienze Naturali.*
Atti. Vol. XXIV-XXXII, 1881-90. 8°.
MODENA.—*Regia Accademia delle Scienze, Lettere ed Arti.*
Memorie. Serie II. Tomo V, VI, 1887-88. 4°.
——*Società dei Naturalisti.*
Memorie. Ser. III. Vol. VII. 1, VIII, IX. 1, 1888-90. 8°.
MONTPELLIER.—*Académie des Sciences et Lettres.*
Mémoires. Section des lettres. Tome VIII. 2, 3, 1888-89. 4°.
MOSCOU.—*Société Impériale des Naturalistes.*
Bulletin. Année 1888, 1889, 1890. i. 8°.
Nouveaux mémoires. Tome XV. 6, 1889. 4°.
Meteorologische Beobachtungen am Observatorium der landwirth. Akademie bei Moskau. Jahr. 1887. ii, 1888, 1889. i. 4°.
MÜNCHEN.—*Kön. bayerische Akademie der Wissenschaften.*
Sitzungsberichte. Philosph.-philolog. und histor. Classe. 1888, 1889, 1890. Bd. I. 1, 2. 8°.
——Mathemat.-physikal. Classe. 1888, 1889, 1890 Heft 1, 2. 8°.
Ueber die Molekularbeschaffenheit der Krystalle. Festrede von Dr. Paul Groth. 1888. 4°.
Ueber die historische Methode auf dem Gebiet des deutschen Civilprocessrechts. Festrede von J. W. v Planck. 1889. 4°.
Georg Simon Ohm's wissenschaftliche Leistungen. Festrede von Eugen Lommel. 1889. 4°.
Die Anfänge einer politischen Literatur bei den Griechen. Festrede von Rudolph Schöll. 1890. 4°.
Gedächtnisrede auf Karl von Prantl. Von W. v. Christ. 1889. 4°.
Gedächtnisrede auf J. von Döllinger. Von C. A. Cornelius. 1890. 4°.
——*Kön. Sternwarte.*
Neue Annalen. Bd. I, 1890. 4°.
MÜNSTER.—*Westfälischer Provincial-Verein für Wissenschaft und Kunst.*
Jahresbericht. XVI, XVII, 1887-88. 8°.
NANCY.—*Académie de Stanislas.*
Mémoires. 5e sér. Tome V-VII, 1888-89. 8°.
NAPOLI.—*R. Accademia delle Scienze Fisiche e Matematiche.*
Atti. Ser. II. Vol. I-III, 1887-89. 4°.
Rediconto. Ser. II. Vol. I-III, 1887-89. 4°.
NEUCHATEL.—*Société des Sciences Naturelles.*
Bulletin. Tome XVI, 1888. 8°.

NEWCASTLE-UPON-TYNE.—*North of England Institute of Mining and Mechanical Engineers.*

Transactions. Vol. XXXVII. 5, 6, XXXVIII. 1–5, 1888–90. 8°.

Report of the French commission on the use of explosives in the presence of fire-damp in mines. Pt. 1, 2. 1890. 8°.

NÜRNBERG.—*Naturhistorische Gesellschaft.*

Jahresbericht. 1888, nebst Abhandlungen, Bd. VIII. 8°.

ODESSA.—*Société des Naturalistes de la Nouvelle Russie.*

Zapiski. Tom. XII. 2, XIII, XIV, 1888–89. 4°.

Matematicheskoe otdielenie. Tom. VIII–X, 1888–89. 8°.

OTTAWA.—*Geological and Natural History Survey of Canada.*

Annual report. New series. Vol. III, with maps, 1887–88. 8°.

Contributions to palæontology. Vol. I. 2. 1889. 8°.

———*Meteorological Service of the Dominion of Canada.*

Report. 1885. 8°.

OXFORD.—*Radcliffe Library.*

Catalogue of books added during 1888, 1889. 8°.

———*Radcliffe Observatory.*

Results of astronomical and meteorological observations. Vol. XLIII, XLIV, 1885, 1886. 8°.

PALERMO.—*R. Accademia di Scienze, Lettere e Belle Arti.*

Atti. Nuova serie. Vol. X, 1888–89. 4°.

PARIS.—*École Normale Supérieure.*

Annales scientifiques. 3e sér. Tome V. 8–12, VI, VII. 1–10, 1888–90. 4°.

———*École Polytechnique.*

Journal. Cahier LVIII, LIX, 1889. 4°.

———*Observatoire.*

Rapport annuel. 1888, 1889. 4°.

———*Société Nationale d'Acclimatation.*

Revue des sciences naturelles appliquées. 4e série. Tome V. 16–24, VI, VII. 1888–90. 8°.

———*Société Géologique de France.*

Bulletin. 3e sér. Tome XIV. 9, XV. 9, XVI. 4–11, XVII. 1–9, XVIII. 1–4, 1886–90. 8°.

———*Société Mathématique de France.*

Bulletin. Tome XVI. 4, XVII, XVIII, 1888–90. 8°.

PENZANCE.—*Royal Geological Society of Cornwall.*

Transactions. Vol. XI. 3, 4, 1888–90. 8°.

PERUGIA.—*Accademia Medico-Chirurgica.*

Atti e rendiconti. Vol. I. 1–4, 1889. 8°.

PISA.—*Società Toscana di Scienze Naturali.*

Memorie. Vol. IX, X, 1888–89. 8°.

Processi verbali. Vol. VI, pp. 105–302, VII, pp. 1–170, 1888–90. 8°.

Commemorazione di Giuseppe Menenghini fatta nell' aula magna dell' Università Pisana al 24 Marzo, 1889. 8°.

POTSDAM.—*Astrophysikalisches Observatorium.*

Publicationen. Bd. IV. 2, VI, 1889. 4°.

PRAG.—*Kön. böhmische Gesellschaft der Wissenschaften.*

Abhandlungen der math.-naturwiss. Classe. Folge VII. Bd. II, III, 1888–90. 4°.

Sitzungsberichte. 1888, 1889, 1890. 8°.

Jahresbericht. 1887–89. 8°.

———*K. k. Sternwarte.*

Magnetische und meteorologische Beobachtungen. Jahrg. XLIX, L, 1888–89. 4°.

Astronomische Beobachtungen. Appendix zu Jahrg. XLVI–XLVIII. 1890. 4°.

PULKOVA.—*Nicolai-Hauptsternwarte.*
Jahresbericht. 1887. 8°.
Tabulae quantitatum Besselianarum, 1890–94. 8°.
QUEBEC.—*Literary and Historical Society.*
Transactions. No. 19, 1887–89. 8°.
REGENSBURG.—*Naturwissenschaftlicher Verein.*
Berichte. Heft I, 1886–87. 8°.
———*Historischer Verein von Oberpfalz und Regensburg.*
Verhandlungen. Bd. XLI, XLII, 1887–88. 8°.
RICHMOND, SURREY.—*Kew Observatory.*
Report of the committee. 1888, 1889. 8°.
RIGA.—*Naturforscher Verein.*
Correspondenzblatt. Jahrg. XXXI–XXXIII, 1888–90. 8°.
LA ROCHELLE.—*Académie des Belles-Lettres, Sciences et Arts.*
Annales de la société des sciences naturelles de la Charente-Inférieure. Vol. XXIV, XXV, 1887–88. 8°.
ROMA.—*Biblioteca Nazionale Centrale Vittorio Emanuele.*
Bollettino delle opere moderne straniere acquistate dalle biblioteche pubbliche governative del regno d'Italia. Vol. III, IV, V. 1–3, 1888–90. 8°.
———*Reale Accademia dei Lincei.*
Atti. Ser. IV. Rendiconti. Vol. IV. i. 11–13, ii, V, VI. i, ii. 1–8, 1888–90. 4°.
———Memorie della classe di scienze morali, storiche e filologiche. Vol. II, III, V, 1886–88. 4°.
———Memorie della classe di scienze fisiche, matematiche e naturali. Vol. III–V, 1886–88. 4°.
———*Accademia Pontifica de' Nuovi Lincei.*
Atti. Tomo XLII, XLIII. 1–3, 1888–90. 4°.
———*Reale Comitato Geologico d'Italia.*
Bollettino. Vol. XVIII–XX, 1887–89. 8°.
———*Società Italiana delle Scienze.*
Memorie di matematica e di fisica. Ser. III. Tomo IV, V and appendice, VII, 1882–90. 4°.
ST. GALLEN.—*Naturwissenschaftliche Gesellschaft.*
Bericht. Jahrg. 1886–87, 1887–88. 8°.
SAN JOSÉ.—*Instituto Meteorologico Nacional.*
Boletin trimestral. No. 4, 1888. 4°.
S. PAOLO.—*Commissao Geographica e Geologica da Provincia de S. Paolo.*
Boletin. No. I–III, 1889. 8°.
ST. PETERSBURG.—*Comité Géologique.*
Mémoires. Vol. II. 2–5, III. 1–4, IV. 1, V. 2–4, VI, VII. 1, 2, VIII. 1, IX. 1, XI. 1, 1885–89. 4°.
Bulletins. Vol. IV. 7–10, V, VII. 1–10, VIII. 1–8, 1885–89. 8°.
Bibliothèque géologique de la Russie. 1885–88. 8°.
———*Hortus Petropolitanus.*
Acta. Tom. X. 2, XI. 1, 1889–90. 8°.
———*Imp. Russ. Geograf. Obshtchestvo.*
Otchet. God 1887–89. 8°.
———*Kais. Akademie der Wissenschaften.*
Bulletin. Tome XXXII. 2–4, XXXIII. 1, 1888–89.
Repertorium der Meteorologie. Bd. XI, XII, 1888–90. 4°.
Neue Reduction der Bradley'schen Beobachtungen, 1750–62. Von A. Auwers. Bd. III, 1888. f°.
ST. PETERSBURG.—*Physikalisches Centralobservatorium.*
Annalen. Jahrg. 1887, 1888, 1889. 4°.

SANTIAGO.—*Deutscher wissenschaftlicher Verein.*
Verhandlungen. Heft VI, 1888. 8°.
*Schweizerische naturforschende Gesellschaft.*
Verhandlungen. Jahresversammlung LXXI, LXXII, 1888–89. 8°.
STOCKHOLM.—*Entomologisk Forening.*
Entomologisk tidskrift. Arg. IX, X, 1888–89. 8°.
———*Kong. Svenska Vetenskaps Akademie.*
Handlingar. Ny följd. Bd. XX, XXI, 1882–85. 4°.
Bihang till handlingar. Bd. IX–XIII, 1884–88. 8°.
Öfversigt af förhandlingar. Arg. XLI–XLV, 1884–88. 8°.
Lefnadsteckningar. Bd. II. 3, 1885. 8°.
Meteorologiska iakttagelser. Bd. XXII–XXVI, 1880–84. 4°.
Förteckning öfver innehålet i skrifter, 1826–1883. 8°.
Ledamöter, 1885–89. 8°.
STUTTGART.—*Verein für vaterländische Naturkunde in Württemberg.*
Jahreshefte. Jahrg. XLV, XLVI, 1889–90. 8°.
SYDNEY.—*Government Observatory.*
Results of meteorological observations made in New South Wales during 1887. 8°.
Results of rain, river and evaporation observations during 1888, 1889. 8°.
———*Linnean Society of New South Wales.*
Proceedings. Series II. Vol. II. 1, 4, 1887–88. 8°.
———*Royal Society of New South Wales.*
Journal and proceedings. Vol. XXII–XXIII, 1888–89. 8°.
TACUBAYA.—*Observatorio Astronomico Nacional.*
Anuario. Año IX–XI, 1889–91. 8°.
THRONDHJEM.—*Kon. Norske Videnskabers Selskab.*
Skrifter. 1886, 1887. 8°.
TIFLIS.—*Physicalisches Observatorium.*
Magnetische Beobachtungen. 1886–87, 1887–88. 8°.
TÔKYÔ.—*Imperial University of Japan.*
Journal of the college of science. Vol. II. 4, 5, III, 1888–90. 4°.
Calendar. 1889–90. 8°.
———*Seismological Society of Japan.*
Transactions. Vol. XIII, 1889. 8°.
TORINO.—*Musei di Zoologia ed Anatomia Comparata.*
Bollettino. No. 49–86, 1888–90. 8°.
TORONTO.—*Canadian Institute.*
Proceedings. Ser. III. Vol. VI, VII, 1889–90. 8°.
Annual report. 1887–88, 1888–89. 8°.
TOULOUSE.—*Académie des Sciences, Inscriptions et Belles-Lettres.*
Mémoires. 8e sér. Tome IX, X. 9e Sér. Tome I. 1887–89. 8°.
TROMSÖ.—*Museum.*
Aarshefter. I–XII, 1878–89. 8°.
Aarsberetning. 1873–1888. 8°.
UPSALA.—*Regia Societas Scientiarum.*
Nova acta. Ser. III. Vol. XIV. 1, 1890. 4°.
Catalogue méthodique des acta et nova acta, 1744–1889. 4°.
UTRECHT.—*Kon. Nederlandsch Meteorologisch Instituut.*
Nederlandsch meteorologisch jaarboek. Jaarg. XXXI. 2, XL, XLI, 1879–90. 4°.
———*Provinciaal Utrechtsch Genootschap van Kunsten en Wetenschappen.*
Verslag van het verhandelde in de algemeene vergadering. 1887–89. 8°.
Aanteekeningen van het verhandelde in de sectie-vergaderingen. 1887–89. 8°.
VENEZIA.—*Istituto Veneto di Scienze, Lettere ed Arti.*
Atti. Ser. VI. Vol. V. 10, VI, VII, 1887–89. 8°.

VICENZA.—*Accademia Olimpica.*
Atti. Vol. XXI, 1886. 8°.
WELLINGTON.—*New Zealand Institute.*
Transactions and proceedings. Vol. XXI, 1888. 8°.
WIEN.—*Kais. Akademie der Wissenschaften.*
Sitzungsberichte. Mathemat.-naturwiss. Classe. Abth. I. Bd. XCV-XCVII, XCVIII. 1-3, 1887-89. 8°.
——*K. k. Central-Anstalt für Meteorologie und Erdmagnetismus.*
Jahrbücher. Neue Folge. XXIV, XXV, 1887-88. 4°.
——*K. k. geologische Reichsanstalt.*
Abhandlungen. Bd. XIII. 1, XV. 1, 2, 1889-90. 4°.
Jahrbuch. Bd. XXXVII. 3, 4, XXXVIII, XXXIX, XL. 1, 2, 1887-90. 8°.
Verhandlungen. Jahrg. 1887, no. 10-18, 1888, 1889, 1890, no. 10-13. 8°.
——*K. k. naturhistorisches Hofmuseum.*
Annalen. Bd. III. 3, 4, IV. 1, 4, V. 1-3, 1888-90. 8°.
——*K. k. Universitäts-Sternwarte.*
Annalen. Bd. V, VI, 1885-86. 4°.
——*K. k. zoologisch-botanische Gesellschaft.*
Verhandlungen. Bd. XXXVIII, XXXIX, XL. 1, 2. 1887. 8°
WIESBADEN.—*Nassauischer Verein für Naturkunde.*
Jahrbücher. Jahrg. XLI, XLII, 1888-89. 8°.
WÜRZBURG.—*Physikalisch-medicinische Gesellschaft.*
Sitzungsberichte. Jahrg. 1888, 1889. 8°.
ZÜRICH.—*Naturforschende Gesellschaft.*
Vierteljahrschrift. Jahrg. XXXI. 3, 4, XXXII, XXXIII, XXXIV. 1, 2, 1886-89. 8°.

---

Recent discussions on the abolition of patents for inventions. Lond. 1869. 8°.
*From R. A. Macfie, Esq.*
De la mesure de la simplicité dans les sciences mathématiques. Par E. Lemoine. Paris, 1888. 8°.
Notes sur diverses questions de la géométrie du triangle. Par E. Lemoine. Paris, 1888. 8°. *From the Author.*
Atmospheric economy of solar radiation. By Arthur Searle. 8°. (From Proc. Amer. Acad. of Arts and Sci., 1888.) *From the Author.*
Eskimo of Hudson's Strait. By F. F. Payne. 8°. (From Proc. Canad. Inst. 1889.) *From the Author.*
Echinoderms from the northern coast of Yucatan and the harbor of Vera Cruz. By J. E. Ives. 8°. (From Proc. Acad. Nat. Sci. of Philad., 1890.)
*From the Author.*
Ueber Feuerbestattung. Vortrag von Prof. Dr. Friedrich Goppelsroeder. Mülhausen, 1890. 8°. *From the Author.*
Die geologischen Horizonte der fossilen Kohlen. Von C. F. Zincken. Leipzig. 1884. 8°. *From the Author*

## I.—Some Experiments on the Physiological Action of Uranium Salts. By R. H. Chittenden and Alexander Lambert, M.D.

In 1885, experiments were commenced in the laboratory of physiological chemistry at Yale University, to ascertain something regarding the physiological and toxical action of uranium salts. At that time there was little accurate knowledge concerning uranium. Gmelin* had, in 1824, performed a few experiments with the nitrate from which he concluded that this salt is a feeble poison; thus he states that 15 grains had no effect on a dog, that 1 drachm merely caused vomiting after more than an hour's interval, and that 34 grains killed a rabbit in 52 hours by stopping the irritability of the heart, while 3 grains injected into the jugular vein of a rabbit caused instant death. Later, in 1851, there appeared a statement in the British and Foreign Medico-chirurgical Review that Leconte always found sugar in the urine of dogs slowly poisoned by small doses of uranium nitrate. This statement was commented upon by Hughes in his manual of pharmico dynamics (p. 866), and has been made the basis of a claim by the so-called homœopathic school that uranium nitrate is a remedy for diabetes. Hughes also refers† to a monograph by Edward Blake on uranium, where three persons and nineteen animals were experimented on. In none of Blake's subjects, however, human or brute, was sugar eliminated in the urine. Ulceration of the pyloric end of the stomach and of the duodenum was found well marked in several of the animals, although in no case was the drug introduced directly into the stomach. Hughes, likewise, refers to several cases of diabetes which he considers were cured by the exhibition of small doses of uranium nitrate,‡ one-sixth to one-third of a grain three times a day. This constitutes all the matter bearing on uranium that we have been able to find.

Our work was commenced by a series of experiments on the influence of a variety of soluble uranium salts on the action of the amylolytic and proteolytic ferments occurring in the animal organ-

* Edinburgh Medical and Surgical Journal, vol. xxvi, p. 136.

† Ibid., p. 867.

‡ Lancet, June 13, 1874.

ism. The results, already published,* show plainly that all of the uranyl salts, with one or two exceptions, have a more or less marked inhibitory influence on amylolytic and proteolytic action. With the salivary ferment, even 0·010 per cent. of uranyl nitrate was sufficient to completely stop all action, while with pepsin-hydrochloric acid and with alkaline trypsin solutions, 0·5 per cent. of the same salt was required to produce an equal effect. In this case the inhibitory action of the uranium salt was, in part at least, due to the formation of a more or less definite and indigestible compound of uranium with the proteid matter to be digested.†

On the excretion of carbonic acid, uranyl nitrate, by a later series of experiments,‡ was also found to have a marked influence. With rabbits, the hypodermic injection of this salt was followed by a slight rise in body temperature and a decided increase in the elimination of carbonic acid. The action of the salt was somewhat slow, but repetition of the experiment always led to an increase of body temperature and a decided increase in the amount of carbonic acid excreted. 0·7 gram of the salt in divided doses was required to produce the result stated, the rabbit not suffering any apparent ill effects from this quantity.

The object of the present series of experiments has been: 1st, to ascertain the influence of uranium salts on proteid metabolism; 2d, to ascertain something regarding the toxic action of uranium salts; and 3d, whether uranium has any influence on the production of glycosuria.

### *Influence on proteid metabolism.*

In this experiment a mongrel bitch weighing 18·8 kilos was employed. The animal was confined in a convenient cage suitably arranged for the collection of the excreta, and was fed during the experiment upon a constant diet of known composition. A large quantity of fresh, lean beef finely chopped, was dessicated at a low temperature until it had lost about 75 per cent. of water. It then contained 11·88 per cent. of nitrogen, as determined by Kjeldahl's method. A large quantity of ordinary soda crackers were obtained and when sampled were found to contain 0·69 per cent. of nitrogen. 40 grams of this prepared beef and 25 grams of the crackers, with

* Chittenden and Hutchinson. Studies from the Laboratory of Physiological Chemistry, Yale University, vol. ii.

† See Chittenden and Whitehouse. Studies, vol. ii, p. 111.

‡ Chittenden and Cummins. Studies, vol. ii.

400 c. c. of water were fed to the animal twice daily, making a total daily income of 10·054 grams of nitrogen. As soon as nitrogenous equilibrium was established, the 24 hours' urine was analyzed for nine consecutive days, thus giving the average composition of the normal excretion. Uranyl nitrate was then administered for ten consecutive days in gradually increasing quantities.

The accompanying tables give the analytical results. Nitrogen was determined by the Kjeldahl method, sulphur and phosphorus by fusion of a given volume of the urine with potassium hydroxide and potassium nitrate in a silver crucible, and precipitation of the sulphur as barium sulphate and of the phosphorus first as phosphoammonium molybdate and then as ammonio-magnesium phosphate.*

NORMAL URINE—Without uranium.

| Date | Volume. | Reaction. | Sp. Gr. | Nitrogen | Sulphur. | Phosphorus. | Dose of Uranyl nitrate. |
|---|---|---|---|---|---|---|---|
| May | c. c. | | | grams. | gram. | gram. | |
| 31 | 565 | acid | 1016 | 8·414 | 0·441 | 0·572 | 0 |
| June | | | | | | | |
| 1 | 610 | " | 1017 | 10·380 | 0·516 | 0·637 | 0 |
| 2 | 630 | " | 1016 | 10·040 | 0·558 | 0·665 | 0 |
| 3 | 600 | " | 1018 | 9·618 | 0·589 | 0·595 | 0 |
| 4 | 630 | " | 1018 | 10·770 | 0·661 | 0·715 | 0 |
| 5 | 570 | " | 1019 | 10·325 | 0·608 | 0·632 | 0 |
| 6 | 460 | " | 1020 | 8·738 | 0·628 | 0·746 | 0 |
| 7 | 590 | " | 1018 | 10·091 | 0·576 | 0·712 | 0 |
| 8 | 640 | " | 1016 | 10·763 | 0·604 | 0·645 | 0·025 gram. 0·025 |
| Total | *5295* | .... | .... | *89·139* | *5·181* | *5·919* | |
| Daily average | *588* | .... | *1017·5* | *9·904* | *0·575* | *0·657* | |

Examination of the first table shows that the animal was in nitrogenous equilibrium, the average daily excretion of nitrogen for the normal period being 9·904 grams while the daily amount of nitrogen taken was 10·054 grams, thus showing a fairly close agreement, espe

* See studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p 88.

cially as there would be a slight loss of nitrogen through the hair shed daily. On commencing the administration of uranium nitrate, small doses were at first given two or three times daily in gelatin capsules, at times not to interfere with digestion. On the second day, uranium was detected in the urine. On June 12th, the 5th day the uranium salt was given, a trace of albumin appeared in the urine and on the day following, the 24 hours' urine contained 1·74 grams of albumin.

WITH URANIUM.

| Date. | Volume. | Reaction. | Sp. Gr. | Total nitrogen. | Nitrogen—nitrogen of albumin. | Sulphur. | Phosphorus. | Dose of Uranyl nitrate. |
|---|---|---|---|---|---|---|---|---|
| June | c. c. | | | grams. | grams. | gram. | gram. | gram. |
| 9 | 600 | acid | 1017 | 10·285 | 10·285 | 0·587 | 1·226 | 0·035<br>0·035 |
| 10 | 600 | " | 1015 | 8·895 | 8·895 | 0·404 | 0·556 | 0·050<br>0·025 |
| 11 | 602 | " | 1018 | 8·925 | 8·925 | 0·574 | 0·612 | 0·050<br>0·025 |
| 12 | 680 | " | 1017 | 8·380 | 8·380 | 0·525 | 0·603 | 0·050 |
| 13 | 640 | " | 1022 | 11·391 | 11·093 | 0·724 | 0·621 | 0·025<br>0·025<br>0·025 |
| 14 | 710 | " | 1020 | 8·847 | 8·439 | 0·634 | 0·628 | 0·025<br>0·050 |
| 15 | 720 | " | 1023 | 10·608 | 10·141 | 0·571 | 0·628 | 0·050<br>0·050<br>0·050 |
| 16 | 830 | " | 1022 | 11·767 | 11·446 | 0·738 | 0·704 | 0·075<br>0·075<br>0·075 |
| 17 | 640 | " | 1025 | 11·178 | 10·955 | 0·606 | 0·626 | 0·150<br>0·150<br>0·150 |
| Total. | *6022* | | | *90·276* | *88·559* | *5·363* | *6·204* | *1·295* grams. |
| Daily average | *669* | | *1019·9* | *10·031* | *9·839* | *0·596* | *0·689* | |
| 18 | 650 | acid | 1026 | 10·179 | 9·772 | 0·611 | 0·542 | 0 |

On the 16th of June, the urine contained 7·7 grams of sugar. The appearance and disappearance of both albumin and sugar were followed quantitatively and the results will be discussed later on. Naturally, the appearance of albumin in the urine compels a correction in the table of nitrogen results on those days when albumin was excreted. This has been done by subtracting from the total nitrogen found, the nitrogen equivalent to the albumin, on the assumption that the latter contains 15·5 per cent. of nitrogen.

Examination of the analytical results shows that the uranium salt has a marked influence on the excretion of water, the increase amounting on an average for the nine days period to over 80 c. c. per day. There is also a very marked increase in the specific gravity of the daily excretion, but this is without doubt in great part due to the presence of albumin and sugar, as the increase is most noticeable on those days when the largest amounts of sugar and albumin were excreted.

Regarding nitrogen, a comparison of the total amounts excreted for the two periods, after correction for the nitrogen of the albumin, indicates that small doses of the uranium salt have little, if any, influence on proteid metabolism. On the last three days of the uranium period, however, when the amount administered had not only been increased, but there was doubtless also an accumulative effect, the excretion of nitrogen appeared to be considerably above the normal, certainly enough to warrant the assumption that comparatively large doses of uranium salts may increase somewhat proteid metabolism, and this view is sustained by the increase in both the total and daily average amounts of sulphur and phosphorus excreted. During the uranium period of ten days, 1·295 grams of the nitrate, or 19·98 grains, were given without any apparent ill effects being immediately produced.

### *Excretion of sugar and albumin.*

After about 0·4 of a gram of uranium nitrate had been given, the urine began to show traces of albumin and five days after this, sugar made its appearance. The amounts excreted are shown in the accompanying table. Sugar was determined by Allihn's gravimetric method, and albumin by boiling with acetic acid and collecting the coagulum on a weighed filter. On the 18th, the weighed diet was discontinued, but the urine was kept under close scrutiny with the results shown in the table. The sugar first disappeared and four days after this the albumin, likewise. On again administering uranium, and in much larger doses than in the first series, both sugar and albumin failed to appear until after a single dose of over 4 grams of the salt had been administered, when considerable albumin showed itself in the urine. We were then compelled to stop the experiment, and the dog was chloroformed and a post-mortem immediately made.

The liver was excessively congested and appeared abnormally large. Microscopic examination of hardened sections showed an

TABLE SHOWING THE AMOUNTS OF ALBUMIN AND SUGAR IN THE DOG'S URINE.

| Date. | Volume. | Reaction. | Sp. Gr. | Albumin. | Sugar. | Dose of Uranyl nitrate. |
|---|---|---|---|---|---|---|
| June | c. c. | | | grams. | grams. | grams. |
| 8 | 640 | acid | 1016 | 0 | 0 | 0·050 |
| 9 | 600 | " | 1017 | 0 | 0 | 0·070 |
| 10 | 600 | " | 1015 | 0 | 0 | 0·075 |
| 11 | 602 | " | 1018 | 0 | 0 | 0·075 |
| 12 | 680 | " | 1017 | trace | 0 | 0·050 |
| 13 | 640 | " | 1022 | 1·741 | 0 | 0·075 |
| 14 | 710 | " | 1020 | 2·634 | 0 | 0·050 |
| 15 | 720 | " | 1023 | 3·009 | 0 | 0·150 |
| 16 | 830 | " | 1022 | 2·066 | 7·735 | 0·225 |
| 17 | 640 | " | 1025 | 1·441 | 7·449 | 0·450 |
| 18 | 650 | " | 1026 | 2·633 | 7·476 | 0 |
| | | weighed | diet dis | continued. | | |
| 19 | 1280 | acid | 1016 | 3·604 | 14·387 | 0 |
| 20 | .... | " | .... | considerable | considerable | 0 |
| 21* | 750 | " | 1027 | 3·048 | 0·525 | 0 |
| 22 | 410 | alkaline | 1035 | 1·756 | 0 | 0 |
| 23† | .... | acid | .... | some | 0 | 0 |
| 24 | 460 | " | 1018 | 0·578 | 0 | 0 |
| 25 | 430 | alkaline | 1030 | trace | 0 | 0 |
| 26 | .... | .... | .... | 0 | 0 | 0 |
| 27 | .... | .... | .... | 0 | 0 | 0·4 |
| 28 | .... | .... | .... | 0 | 0 | 0·5 |
| 29 | .... | .... | .... | 0 | 0 | 0 |
| 30 | .... | .... | .... | 0 | 0 | 0 |
| July 1 | .... | .... | .... | 0 | 0 | 1·0 |
| 2 | .... | .... | .... | 0 | 0 | 0 |
| 3 | .... | .... | .... | 0 | 0 | 1·5 |
| 4 | .... | .... | .... | 0 | 0 | 4·4 |
| 5 | .... | .... | .... | considerable | 0 | 0 |

infiltration of cells around the blood vessels, but aside from this and an apparent tendency of the liver cells to separate into stringy masses there was nothing abnormal. The kidneys both had adherent capsules and the medullary portion was large in ratio to the cortex, which, besides being smaller than normal, was striated. The lesion as made out microscopically was acute parenchymatous neph-

* On this day granular casts were found, but they were not present on the following day.

† Dog vomited freely.

ritis. The endothelial cells of the malphigian tufts were swollen and proliferated, so that frequently the tuft of vessels was compressed by these proliferating cells and by the detritus and infiltrating pus cells. In the convoluted tubules the cells were swollen, granular and very much broken down, while the lumen of the tubes themselves was often filled with detritus and cast matter. In the straight tubules, the cells were also occasionally broken down and frequently swollen and granular. The stroma was found in places infiltrated with pus cells. Stomach and intestines were normal.

Further data regarding the excretion of albumin and sugar, through the action of uranium, are given in the description of the toxic action of this substance.

### *The toxic action of uranium.*

Our experiments on the toxic action of uranium have been confined wholly to the action of pure uranyl nitrate on rabbits, nine in number. The animals were confined in cages with proper outlets for collecting the excreta, and were fed on grass, spinach, and other green food.

### *Experiment I.*

Large, vigorous buck. In this experiment small doses of uranium, gradually increased, were given in gelatin capsules, and the urine examined each day for albumin and sugar. The accompanying table gives the details of dose and the amounts of sugar and albumin found.

Outside of changes in the urine there were no indications of toxic action until on the ninth day, when a slight weakness was noticeable, especially in the hind legs. After this, the animal gradually grew weaker and emaciated, with total loss of appetite and with eyes dull and watery. On the 8th of June, a large quantity of thick tenacious mucus mixed with pin-head fæces was passed. Motion of all kinds was difficult. On the 9th of June, the animal could not sit up and during portions of the day there appeared to be paralysis of both hind legs. Later in the day the power of motion returned, and when placed on his back the rabbit could kick out feebly with both legs. On the next day his weakness and dullness were still more pronounced, and when stirred from a squatting posture he trembled violently. His eyes were watery and glazed, with the pupils widely dilated. On touching the cornea he did not offer to close the eyelids, but endeavored to withdraw his head. All power of vision was apparently lost. Later, he lost the power of coördination in his legs, was unable to make any coördinate movements, though still retaining

the power to move each limb separately. All fæcal discharges had stopped and the animal refused all food.

| Date. | Volume. | Reaction. | Sp. Gr. | Albumin. | Sugar. | Dose of Uranyl nitrate. |
|---|---|---|---|---|---|---|
| May | c. c. | | | gram. | gram. | gram. |
| 25 | 73 | alkaline. | 1020 | 0 | 0 | 0·025 |
| 26 | — | " | — | 0 | 0 | 0·050<br>0·025 |
| 27 | 70 | " | 1020 | 0 | 0 | 0·050 |
| 28 | 85 | " | 1016 | 0 | 0·035 | 0·050 |
| 29 | 75 | " | 1022 | 0·189 | trace. | 0·050 |
| 30 | — | — | — | — | — | 0·050 |
| 31 | 75 | " | 1023 | 0·140 | 0·492 | 0·050<br>0·025 |
| June 1 | 82 | " | 1023 | 0·125 | 0 | 0·050<br>0·050 |
| 2 | 75 | " | 1021 | 0·101 | 0 | 0·050<br>0·050 |
| 3 | 80 | " | 1020 | 0·173 | 0 | 0·050<br>0·050 |
| 4 | 18 | neutral. | — | 0·217 | 0 | 0·050<br>0·050 |
| 5 | 12 | alkaline. | — | 0·155 | 0 | 0·050 |
| 6 | — | — | — | — | — | 0·100 |
| 7 | 45 | " | 1025 | 0·518 | 0 | 0·100<br>0·100 |
| 8 | 30 | " | — | 0·301 | 0 | 0·150<br>0·150 |
| 9 | 20 | " | — | considerable. | 0 | 0·200<br>0·200 |
| 10 | 0 | | | | | 0·200<br>0·150 |
| | | | | | | 2.175 grams. |

On the morning of the 11th found dead. Post-mortem showed the heart distended on the right side and the lungs normal. The liver was much smaller than normal and the gall-bladder full of very black bile. On a microscopic examination of the liver, the only noticeable feature was the extreme congestion. In the stomach, the mucous membrane seemed somewhat disintegrated. In the intestines, brown colored spots were seen where Peyer's patches are situated, and microscopic examination showed that the latter were deeply inflamed. Further, from the large amount of mucus in the mucous glands, it was evident that there was acute catarrhal inflammation of the intestines. The cæcum was full of black matter. There were no normal fæces in the colon or rectum, only the pin-head variety.

The kidneys were very much diminished in size and extremely congested. Sections showed that the epithelial cells of the malpighian tufts were not swollen or proliferated, though in some places the tufts of vessels were slightly compressed by detritus. The cells of the convoluted tubules were swollen, granular and broken down or gone entirely, as were also the cells of the straight tubes, the latter being also filled in places with casts and detritus. The vessels between the tubes were filled with blood. The stroma was swollen and infiltrated with pus cells and in places there were extravasations of blood.

Brain and cord were slightly congested, but otherwise normal.

Examination of the table of urine tests shows that sugar appeared first in the urine, but continued only for three days, while albumin showed itself immediately after the first appearance of the sugar and continued throughout the experiment. The amount of urine voided grew gradually smaller as the animal began to feel the full effects of the uranium.

### *Experiment II.*

In this experiment, uranium was given as indicated in the following table, and the urine examined each day.

| Date. | Volume. | Reaction. | Sp Gr. | Albumin. | Sugar. | Dose of uranyl nitrate. |
|---|---|---|---|---|---|---|
| June | c. c. | | | gram. | gram. | gram. |
| 1 | — | alkaline. | — | 0 | 0 | 0·050 |
| 2 | 100 | " | 1014 | trace. | 0 | 0·050<br>0·050 |
| 3 | 55 | " | 1025 | 0·192 | 0 | 0·050<br>0·050 |
| 4 | 41 | " | — | 0·227 | 0·406 | 0·050<br>0·050 |
| 5<br>6 | 125 | acid. | 1024 | 1·095 | 1·078 | 0·050<br>0·050 |
| 7 | 120 | alkaline. | 1024 | 0·523 | 0·917 | 0·050<br>0·050 |
| 8 | 100 | acid. | 1022 | 0·237 | trace. | 0·100<br>0·100 |
| 9 | 55 | alkaline. | — | 0·351 | 0 | 0·100<br>0·100 |
| 10 | 125 | " | 1022 | 0·687 | 0·430 | 0·100<br>0·100 |
| 11 | 75 | " | — | considerable. | 0 | 0·100 |
| 12 | 75 | " | 1022 | 0·332 | 0 | 0·100 |
| | | | | | | 1.350 grams. |

On June 4th, the animal began to show weakness, particularly noticeable in his hind legs, but this disappeared and the animal

appeared better. On the 11th, it had lost its appetite, become emaciated and showed general weakness and loss of muscular power. On the following day at noon it was found dead, without having shown any marked symptoms other than general depression of the muscular system.

The heart was found greatly distended on the right side, lungs normal and the liver small and congested, especially near the lower edges of the left lobe. Kidneys were also small and congested. The bladder contained quite a little urine, with considerable albumin and sugar in it.

### *Experiment III.*

This experiment was practically a duplicate of the preceding. An albino buck was used and the uranium salt was forced down the throat in gelatin capsules, as in the preceding experiments. The table shows the amount of nitrate given and the character of the urine each day.

| Date. | Volume. | Reaction. | Sp. Gr. | Albumin. | Sugar. | Dose of uranyl nitrate |
|---|---|---|---|---|---|---|
| May | c. c. | | | gram. | gram. | gram. |
| 25 | 171 | alkaline. | 1014 | 0 | 0 | 0·025 |
| 26 | — | acid. | — | trace. | 0 | 0·050<br>0·025 |
| 27<br>28 | no urine passed. | | | | | 0·050 |
| 29<br>30 | 75 | alkaline. | 1026 | 0·494 | 0.275 | 0·050<br>0·050 |
| 31 | no urine passed. | | | | | 0·050<br>0·025 |
| | | | | | | 0·300 gram. |

The first symptom noticeable was the suppression of urine for over forty-eight hours. On the 27th, the animal was quite dull and showed signs of general weakness. As he walked, he moved as if it caused him pain in the lumbar region. On the next day he showed great thirst, and he finally died on June 1st, a loose diarrhœa setting in a few hours before death.

Post-mortem examination showed that the heart had stopped in diastole, and that it was much distended with blood. Lungs were normal. The liver was congested, as were also the kidneys. The latter had non-adherent capsules. The stomach contained considerable undigested food, and its mucous membrane was partly disintegrated. Portions of the duodenum were somewhat congested. The

bladder contained a few cubic centimeters of urine, which had in it considerable sugar and some albumin.

The most noticeable feature of this experiment was the suppression of urine.

### *Experiment IV.*

In this experiment, a large white buck was used and the uranium was administered in capsules as follows:

| | | | | |
|---|---|---|---|---|
| May 17 | 0·050 gram | uranyl | nitrate. | |
| 18 | 0·075 | " | " | " |
| 19 | 0·075 | " | " | " |
| 20 | 0·025 | " | " | " |
| | 0·225 | | | |

There were no symptoms of toxic action until the morning of the 20th. The rabbit was then dull and weak. He trembled violently as he hopped about; the hind legs appeared partly paralyzed. The pupils were dilated and the appetite gone. On the 21st, weakness was still more pronounced. Any movement was accompanied by tremblings and great difficulty was experienced while walking, in making the different movements correctly. There was severe diarrhœa and the animal showed extreme emaciation. On the 22d, the diarrhœa had passed into an involuntary defecation more or less continuous. The animal was too weak to move and lay all the forenoon breathing heavily, though the number of respirations per minute did not go much beyond normal, 45–54 per minute. On the afternoon of this day, two days after the last dose of uranium had been administered, the animal appeared stronger, the involuntary defecations had ceased, though a loose diarrhœa still continued, and the animal appeared to have recovered the use of its locomotive muscles. On the 23d, however, the animal was again unable to move and finally died at noon, the diarrhœa having continued more or less up to death.

During the last three days there was complete suppression of urine, but on the 20th, 59 c. c. of urine were passed, of specific gravity 1022, and which contained 0·656 gram of sugar.

On post-mortem examination, the heart was found to have stopped in diastole and engorged with blood. The lungs were congested. The kidneys had non-adherent capsules and the cortex and medulla showed severe congestion. The stomach contained no food, but considerable tenacious mucus. The small intestines from duodenum to cæcum were very much congested.

### *Experiment V.*

Gray and white buck. This experiment was a repetition of No. IV, the same amounts of uranium salt being given and at the same intervals of time. The animal died three days after the last dose of uranium had been administered. The symptoms were not very marked, simply loss of muscular power, and a gradual wasting away. On post mortem, the heart was found in diastole and engorged with blood. Lungs congested. The liver was black with the blood in it, especially on the edges, and when pressed between the fingers was extremely pliable and of a soft, pulpy consistency, showing marked parenchymatous degeneration. The gall bladder was distended with very dark bile. Both kidneys had non-adherent capsules. The left kidney was slightly congested. The intestines had an inflamed patch in the jejunum and were congested for a foot above it. The bladder contained a little urine, which gave reactions for both sugar and albumin. Sugar and albumin were also found in the urine several days before death.

### *Experiment VI.*

In this experiment still smaller amounts of uranium were administered, a total of only 0·175 gram being given in three days. The following table shows the dosage and the changes in the urine:

| Date. | Volume. | Reaction. | Sp. Gr. | Albumin. | Sugar. | Dose of uranyl nitrate. |
|---|---|---|---|---|---|---|
| June | c. c. | | | gram. | gram. | gram. |
| 14 | — | | | 0 | 0 | 0·050 |
| 15 | — | alkaline | 1043 | 0 | 0 | 0·050<br>0·025 |
| 16 | 110 | " | 1027 | 0·104 | 0 | 0·050 |
| 17 | 75 | " | 1022 | 0·337 | 0 | |
| 18 | 30 | " | — | 0·126 | 0 | 0·175 |
| 19<br>20 | 60 | " | — | 0·154 | 0 | |
| 21 | — | " | — | some | 0 | |
| 22 | 50 | " | 1015 | trace | 0 | |

There were no noticeable symptoms of toxic action aside from the changes in the urine, and even here there was no sugar at any time present. Just as the albumin had almost disappeared from the urine and we looked for speedy recovery, the animal was found dead (on the 23d). Post-mortem showed the heart in diastole, engorged with blood. Lungs normal. Liver normal, aside from a slight congestion.

Sections under the microscope showed no pathological changes. The kidneys were congested. Under the microscope, the epithelial cells were proliferated and occasionally the tufts were seen slightly compressed by these proliferating cells and detritus. The convoluted tubules showed swollen and granular cells, even broken down, in places. In the straight tubules, the cells were also swollen and granular and sometimes were detached from the tubes. In places, there was cast matter and detritus in the tubes. The stroma was normal. Hence, here, as in many of the preceding experiments, it is a case of acute parenchymatous nephritis. The stomach was full of undigested food and apparently normal; the duodenum was congested and the small intestines throughout were nearly empty, except for a little mucus. The bladder had in it about 10 c. c. of urine, which contained a trace of albumin but no sugar.

### *Experiment VII.*

An albino rabbit. A single dose of 0·3 gram of uranyl nitrate was given in a gelatin capsule by mouth on June 13th. Outside of changes in the urine there were no symptoms whatever until the 17th, when in the early morning the animal was found weak and powerless, all motor power completely gone. It rapidly grew weaker and died in the afternoon of the same day.

The right side of the heart was found much distended. The lungs were normal, the liver small and congested. Kidneys were also small and congested and of a cloudy appearance. Microscopic examination showed acute parenchymatous nephritis. Stomach was full of undigested food, but the intestines were empty. Both appeared normal.

On June 15th, 160 c. c. of alkaline urine were passed, of specific gravity 1023. It contained 0·748 gram of albumin and 1·069 grams of sugar. On the 16th, 30 c. c. were passed of 1022 specific gravity and containing 0·412 gram of albumin and 0·354 gram of sugar. On the 17th, no urine was passed and on making the post mortem the bladder was found empty.

With this rabbit, an attempt was made to ascertain how much carbohydrate matter there was in the liver at the time of death. 40 grams of the sampled and finely ground liver were thoroughly extracted with boiling water (continuous extraction for three days), frequently replaced. The several decoctions were ultimately united and finally brought to a volume of 500 c. c. Two portions of 200 c. c. each were placed in suitable flasks and sufficient hydrochloric acid

added to each, to make the fluid contain 2 per cent. HCl. The acid fluids were then heated on water-baths for 15 hours, in order to convert all carbohydrate matter into dextrose. The fluids were then neutralized, evaporated and finally tested for dextrose with Fehling's solution, by Allihn's gravimetric method. Both solutions failed to give any reducing action whatever, thus showing a total lack of carbohydrate matter in the liver. This is in strong contrast to the normal condition of a rabbit's liver, which contains an abundance of carbohydrate matter, both sugar and glycogen; on an average 10·35 per cent. of total carbohydrates, as determined by methods similar to the one just described.* Glycogenic function is then destroyed by uranium, the same as in phosphorus poisoning, but unlike the action of phosphorus there is apparently no fatty degeneration of the organs.

The liver here experimented with was small for the size of the rabbit, a large white buck. It was also noticeable, as in many of the other rabbits experimented on, that while the stomach was full of food, sometimes even distended by it, there was nothing at all in the intestines below the pylorus until the cæcum was reached, which was again full, and the intestines below were either almost free from fæces or else contained only the pin-head variety. The food in the stomach was wholly undigested. As has been already pointed out, a small percentage of a soluble uranium salt is sufficient to completely stop gastric and pancreatic digestion. This being the case it is quite probable that the emaciation, etc., so noticeable in uranium poisoning, is the direct result of the action of the salt on the digestive functions. Nothing being digested there would be no matter for absorption, and hence no sugar-forming material for the liver. All the carbohydrate matter stored up would in a little time be completely consumed and as the portal blood could bring no new nutritive matter, the liver would naturally diminish in size and the animal become emaciated and eventually die from that cause alone, even if the uranium gave no other direct cause of death. In this connection we also need to recall the general increased metabolism of both nitrogenous and non-nitrogenous matter, under the influence of uranium.

The uranium salt may also act specifically on the liver cells, affecting their metabolic power, preventing any storage of carbohydrate matter or more probably causing a degeneration of the cells, by which they may be led to give up to the blood in abnormal abundance all the carbohydrate matter previously stored up. This possible

* Post-mortem formation of sugar in the liver. Studies from Laboratory of Physiological Chemistry, Yale University, vol. 1, p. 171.

specific action of the poison on the liver cells would in a measure explain the temporary glycosuria, which appears and then disappears so frequently in uranium poisoning. Further, by a combination of this specific action with the non-absorption of nutritive matter through retarded digestion, it would be easy to explain the alternate appearance, disappearance and re-appearance, of sugar in the urine in these cases. Where the symptoms run their course with a fair degree of rapidity, as in continued dosing with uranium, sugar appears in the urine for a few days and then disappears, although the amount of uranium administered may be steadily increased. In fact, the increased dose of uranium is doubtless the cause of the further non-appearance of the sugar, since the stored up carbohydrate material having been wholly used up, and at the same time digestion and absorption being prevented, there is no more carbohydrate-producing material available, either directly or indirectly. On the other hand, if, after the sugar at first present has disappeared from the urine, there comes an interval of a day or so when uranium is not administered and digestion again starts up, then the specific action may again come into play and a temporary glycosuria again result. This condition was noticed in experiment No. II.

In some of the experiments, there seems to have been a visible change in the hepatic cells, as in the experiment next to be described, where under the microscope the cell bodies appeared as if collected into small granules or even broken down. Again, in some experiments, as in No. V, there was a noticeable pulpy degeneration of the liver cells.

### *Experiment VIII.*

In this experiment, the uranium salt was introduced by hypodermic injection. 0·23 gram of uranyl nitrate in a little water was injected beneath the skin of the leg of a good-sized rabbit, on June 20th at 4.0 P. M. Two days after he showed marked weakness, although his appetite remained good. On the following day he appeared quite dormant and could be roused only with difficulty; power of motion seemed to be nearly gone. He died the next day at noon. On June 21st, the day following the administration of the uranium, 60 c.c. of alkaline urine were passed containing 0·234 gram of albumin and 0·660 gram of sugar. After this, there was complete suppression of urine till death.

*Post-mortem.*—Heart had stopped in diastole; lungs normal. The liver was mottled and congested slightly in areas. Under the micro-

scope, sections showed the cell bodies strongly granulated, with their outlines mostly clear, but in places broken down.

There was also an infiltration of pus cells around the blood vessels. The kidneys were normal in size and appearance, with non-adherent capsules. Under the microscope, however, all the conditions characteristic of acute parenchymatous nephritis were to be seen. The epithelial cells of the malpighian tufts were proliferated, so much so as to compress the vessels. Both convoluted and straight tubules had their cells swollen, granular and broken down. The stroma was normal. Some adipose tissue in the pelvis of the kidney was infiltrated with pus cells. There was also considerable detritus between the papillæ. The bladder was empty and contracted. In the abdominal cavity there was about 30 c.c. of a coagulable, clear fluid. The stomach was full of food, the intestines empty. No fæces in colon and rectum. No signs of congestion in the alimentary tract, except in the rectum, where there were red blotches which proved to be the beginning of inflammation and infiltration of pus cells. The animal appeared to have died from suppression of urine.

### *Experiment IX.*

In this experiment, one large dose of uranium (1·0 gram of the nitrate) was given by mouth on June 27th, at 4.30 P. M. On July 5th the animal was still alive, but weak and emaciated. There were no symptoms other than those already described. Owing to lack of time we were not able to continue the experiment, but wishing to see any changes which might have occurred in the organs the animal was chloroformed and a post mortem made. On June 28th, the urine passed contained some albumin, but no sugar; on July 1st, considerable albumin, but still no sugar.

The liver was found to be small but not congested. The cell substance, as seen under the microscope, was collected into small granules. Nuclei and nucleoli were quite distinct. Kidneys had non-adherent capsules, but they were congested and the cortex looked striated. Under the microscope, the epithelial cells of the glomeruli were seen to be swollen and proliferated. In the convoluted tubes, the cells were slightly swollen and granular and occasionally broken down. The straight tubes were also broken down in places and contained cast matter. The stroma was normal. Stomach was filled with food, while the intestines were entirely empty. Peyer's patches were swollen and infiltrated with pus cells. The cæcum was partially filled with matter and the end of it looked honey-combed. It

proved to be slightly inflamed, and with cells infiltrated into it more or less. Colon and rectum were empty.

From these results, collectively, it is to be seen that uranium is an irritant poison and, like other metallic irritants, produces gastro-intestinal irritation of more or less intensity, as shown by the acute diarrhœa and other symptoms met with in this form of poisoning. In the majority of cases, the action of the uranium salt on the intestines results in a simple enteritis, but this is liable to pass eventually into acute catarrhal inflammation.

As ordinarily administered, it is not in any sense a rapid poison; the ingestion of a fatal dose of a uranium salt is not followed by any noticeable effects for some time. The action of a small amount (150 milligrams) is apparently as rapid and pronounced as that of large quantities (1 gram). The first noticeable symptom in rabbits is general weakness, lack of motor power, loss of coördination and occasional temporary paralysis of the locomotor muscles. Introduced into the stomach in sufficient amounts, it checks digestion and even stops it altogether. On the other hand, it appears to increase somewhat proteid metabolism and also to increase the elimination of carbonic acid and raise the body temperature. Hence, it is to be considered as having a direct action on nutrition, the disturbance of which is also plainly indicated by the rapid emaciation which follows the administration of uranium.

Its most marked lesions are its destructive action on the kidneys, and its destruction of the kidney tissue itself. It causes here an acute parenchymatous nephritis of the same kind as found in arsenic, mercury, and phosphorus poisoning. Further, the quantity of albumin found in the urine shows plainly how greatly the blood vessels are involved in the inflammation. The albuminuria produced is severe and constant, and when the uranium is given in a single large dose, as in experiment No. IX, or in a small dose by hypodermic injection, as in experiment No. VIII, then albumin may appear in the urine within 24 hours. The uranium must have some specifically destructive action on the kidney epithelium cells, causing them to swell and break down. At first, with small doses of uranium, the urine is decidedly increased in volume, but later on, when toxic action is more pronounced, there may be a partial or even complete suppression of the urine. This latter condition is naturally more quickly produced by large doses of uranium. In several cases, suppression of urine would seem to have been the cause of death. The urine, too, in a short time after the admin-

istration of uranium contains more or less sugar; as a rule the sugar does not make its appearance until after the albumin. Salkowski has shown that mercury will also cause diabetes in rabbits, but it is questionable whether it is as constant a symptom as in uranium poisoning. It is also stated that in phosphorus poisoning the urine sometimes contains sugar.* In the hypodermic injection of uranium, experiment No. VIII, sugar appeared in the rabbit's urine within 24 hours. The production of glycosuria is a very characteristic symptom in uranium poisoning. The urine also contains invariably a large amount of crystallized calcium oxalate, which would also point to decided malnutrition and help explain the marked emaciation so commonly seen.

In those cases where the poisoning becomes in a measure chronic, the nervous symptoms sometimes predominate, as shown in loss of sight and power of coördination.

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* H. C. Wood. Therapeutics, p. 110.

## II.—Elastin and the Elastose Bodies. By R. H. Chittenden and Horace S. Hart, B.A., Ph.B.

Elastin, the basis of the so-called elastic tissue, has generally been considered one of the most indigestible of the albuminoid substances. In fact, the older writers looked on it as almost insoluble in the digestive juices. Recently, however, the experiments of Etzinger,* Horbaczewski,† and Morochowetz,‡ have shown that both the *ligamentum nuchæ* of the ox when finely divided, and purified elastin when powdered, are fairly soluble both in pepsin-hydrochloric acid and in natural gastric juice, as Horbaczewski's observations on a man with stomach fistula have fully indicated.

It has been our object, therefore, to study somewhat in detail the primary cleavage products of elastin, so far as they may be formed, making use of the methods§ which have yielded such fruitful results with many of the proteid bodies.

### *Preparation of Elastin.*

As usually described, elastin is a body free from sulphur, insoluble in water even after several days boiling, likewise in cold dilute alkali, acetic acid, dilute hydrochloric acid, alcohol, and ether. Naturally, therefore, the method of preparing such an insoluble body has consisted in removing from a tissue rich in elastin, all extraneous substances by successive treatment with the above-mentioned reagents. In fact, so vigorous is the method of treatment, as usually described, that it appears almost questionable whether a body belonging to a group noted for ease of decomposition might not suffer some change in such a long process of preparation.

We have employed two methods, the first one of which is practically identical with that followed by Horbaczewski. The neck bands of a number of recently killed oxen were thoroughly freed from all adhering fat and muscle and then chopped quite fine; they weighed

* Zeitschrift für Biologie, Band x, p. 84.

† Ueber das Verhalten des elastins bei der Pepsinverdauung, Zeitschrift für physiologische chemie, Band vi, p. 330.

‡ Verdauungsgesetze, Abstract in Jahresbericht für Thierchemie, 1886, p. 271.

§ Kühne and Chittenden, Zeitschrift für Biologie, Band xx, xxii; also Studies from Laboratory of Physiological Chem. Yale University, vol. ii.

2400 grams. The mass was boiled for four days in water frequently changed, after which it was soaked for 45 hours in 8 litres of 1 per cent. potassium hydroxide, and then boiled with the solution for 4 hours. The tissue was freed from alkali by thorough washing with water, and was then boiled with water, frequently changed, for 16 hours. The potassium hydroxide solution on neutralization, gave a heavy precipitate slightly soluble in excess of 0·4 per cent. hydrochloric acid, easily soluble in strong acid. The addition of acid in excess caused a strong odor of hydrogen sulphide to be given off.

After boiling the tissue a second time with water, it was placed in 8 litres of 10 per cent. acetic acid and warmed for an hour and a half, after which it was allowed to stand in the cold for 16 hours. It was then boiled for 4 hours in the same acetic acid solution, and afterwards washed thoroughly with water. The tissue was then placed in 10 litres of 5 per cent. hydrochloric acid and allowed to soak for some time in the cold, after which it was washed in running water and macerated in water, frequently changed, for 24 hours to take out all traces of the acid. After this treatment, the tissue was boiled with successive portions of water for about 45 hours, when it was placed in a large volume of 95 per cent. alcohol and allowed to remain for several days. It was afterwards boiled in alcohol for about 15 hours and then placed in an excess of ether, after which it was extracted with warm ether for two days.

ANALYSIS OF ELASTIN A.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. | | | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | c. c. | T. °C. | Pressure. mm. | | | |
| I | 0·4250 | 0·2745 | 7·17 | 0·8381 | 53·77 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·3398 | 0·2213 | 7·23 | 0·6697 | 53·74 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5454 | ---- | ---- | ---- | ---- | 72·7 | 8·6 | 771·5 | 16·47 | ---- | ---- |
| IV | 0·4440 | ---- | ---- | ---- | ---- | 59·8 | 9·0 | 771·2 | 16·59 | ---- | ---- |
| V | 0·5125 | ---- | ---- | ---- | ---- | 69·4 | 9·4 | 767·1 | 16·59 | ---- | ---- |
| VI | 0·2971 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0027 | 0·9 |

*Percentage composition of ash-free substance:*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 54·26 | 54·22 | ---- | ---- | ---- | *54·24* |
| H | 7·24 | 7·30 | ---- | ---- | ---- | *7·27* |
| N | ---- | ---- | 16·74 | 16·76 | 16·62 | *16·70* |
| O | ---- | ---- | ---- | ---- | ---- | *21·79* |
| | | | | | | *100·00* |

The tissue on being freed from water was hard, tough, and difficult to powder. It was finally ground to a coarse powder and then re-extracted with warm ether, until the ether on evaporation left no appreciable residue. This took some time, for, as previously pointed out by Horbaczewski, there apparently remains in the tissue a small amount of fat-like matter, which dissolves very slowly in ether and which can be completely extracted only by first grinding the purified tissue as fine as possible. The elastin prepared in this manner we have termed for convenience *Elastin A.*

A portion of the preparation dried at 110° C. gave on analysis the results contained in the accompanying table. The methods of analysis were the same as those previously described in former articles on the proteoses.* By fusion with potassium hydroxide and potassium nitrate, according to the method recommended by Hammarsten,† no sulphur could be found. Elastic tissue, however, unquestionably contains sulphur, but whether it exists there as a constituent part of the elastin molecule, or loosely united as in keratin, or as a constituent of some adhering proteid or other substance, it is difficult to say. Certainly, the boiling of elastic tissue with 1 per cent. potassium hydroxide for several hours might reasonably be expected to remove a part at least of any sulphur which might be present, and if by this process sulphur is removed from the tissue might it not as probably come from the elastin, as from any other proteid substance? Treatment with acid, of the alkaline solution obtained in the preparation of *A*, plainly showed the presence of hydrogen sulphide and we therefore decided in the preparation of the second portion of elastin to omit the treatment with alkali. Accordingly, 1700 grams of carefully cleaned neck bands from oxen were treated in the same manner as in *A*, except that the alkali was omitted, and in its place, treatment of the tissue with both acetic and hydrochloric acid was repeated twice. In subjecting elastin to the action of 5 per cent. hydrochloric acid care must be taken, before boiling the washed tissue with water, to see that every trace of acid is removed, otherwise the faintly acid water formed will at 100° C. give rise to a partial decomposition of the elastin.

After thorough extraction of the powdered elastin with ether, a portion dried at 110° C. gave by analysis the results shown in the following table, *Elastin B.*

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* See Zeitschrift für Biologie, Band xx, p. 11, and Amer. Chem. Jour., vol. vi, p. 3.

† Zeitschrift für physiologische Chemie, Band ix, p. 298.

ANALYSIS OF ELASTIN B.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | Ash found. gram. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5833 | 0·3744 | 7·18 | 1·1531 | 53·90 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·4374 | 0·2851 | 7·24 | 0·8653 | 53·94 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·3836 | ---- | ---- | ---- | ---- | 54·1 | 11·0 | 754·8 | 16·91 | ---- | ---- | ---- |
| IV | 0·4821 | ---- | ---- | ---- | ---- | 66·8 | 11·7 | 760·4 | 16·69 | ---- | ---- | ---- |
| V | 0·9095 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0170 | 0·25 | ---- |
| VI | 0·7854 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0208 | 0·36 | ---- |
| VII | 0·4235 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0015 |
| VIII | 0·4583 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0013 |

*Percentage composition of ash-free substance:*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 54·06 | 54·10 | ---- | ---- | ---- | ---- | *54·08* |
| H | 7·15 | 7·26 | ---- | ---- | ---- | ---- | *7·20* |
| N | ---- | ---- | 16·96 | 16·75 | ---- | ---- | *16·85* |
| S | ---- | ---- | ---- | ---- | 0·34 | 0·29 | *0·30* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *21·57* |
| | | | | | | | *100·00* |

Comparison of the two products shows a fairly close agreement in composition. In *B*, however, the carbon is slightly lower and the nitrogen a trifle higher than in *A*. Further, Elastin *B* contains 0·3 per cent. of sulphur, as determined by the modified Liebig's process.* Müller originally reported elastin, purified, however, by the use of an alkali, as containing 0·08 per cent. of sulphur, but this he considered merely as an impurity.

Morochowetz, who appears to have made a hydration product of elastin by the simple action of heat and water, states that it contains 0·617 per cent. of sulphur, but the details of his process we do not know.† If the figures are correct, his elastin must have probably contained double the percentage of sulphur present in elastin *B*. Whether pure elastin does contain sulphur, or whether the 0·3 per cent. present in preparation *B* is simply a constituent of some adhering proteid, removable by alkali, we are not at present prepared to say, but deem it probable that elastin does contain a small amount of sulphur.

* See studies from Laboratory of Physiological Chemistry, Yale University, vol. ii. p 163.

† We have seen only an abstract in Jahresbericht für Thierchemie für 1886, p. 271.

Elastin *A*, prepared by the usual process, shows a close agreement in composition with Horbaczewski's product, but is quite different from the product obtained by Müller and Tilanus, as seen from the following table.

| | *Elastin A.* | *Horbaczewski.* | *Müller.** | *Tilanus.** |
|---|---|---|---|---|
| C | 54·24 | 54·32 | 55·09–55·72 | 54·90–55·65 |
| H | 7·27 | 6·99 | 7·11–7·67 | 7·25–7·41 |
| N | 16·70 | 16·75 | 15·71–16·52 | 17·52–17·74 |

The higher content of carbon in the preparations of Müller and Tilanus are doubtless due to the presence of more or less fat, not completely extracted by ether.

### *Decomposition of Elastin by acid water at* 100° C.

On heating moist elastin with water containing a trace of hydrochloric acid, for some hours at or near 100° C. it soon commences to swell, and after a time becomes converted into a semi-gelatinous mass, a portion of which, as the boiling continues, becomes soluble and passes into the acid fluid. The liquid gives no reaction with tannic acid for gelatin, but does give a strong biuret reaction and yields a heavy turbidity when excess of strong potassium hydroxide is added to the solution. Evidently, under these conditions, some soluble cleavage product or products are formed, percipitable by excess of caustic alkali.

These products we prepared in quantity by taking a portion of elastin *A*, soaking it for some time in 5 per cent. hydrochloric acid, until the softened elastin was thoroughly impregnated with the acid, then washing out the excess of acid by soaking it for 20 hours in a large volume of water. The elastin, still quite strongly acid to test papers, was then boiled in water for about 10 hours, when the acid liquid was strained off from the gelatinous elastin. The boiling was then continued with a fresh volume of water, the gelatinous mass still containing sufficient acid to render the whole fluid distinctly acid to test papers. This process was repeated until the elastin had been heated for 45 hours with water gradually containing less and less acid, until finally the elastin remaining had entirely lost its former gelatinous appearance and taken on its original look. All of the acid fluids were united and concentrated somewhat,

* Gorup-Besanez, Physiologische Chemie, Dritte Auflage, p. 148

then neutralized with sodium carbonate. No neutralization precipitate was observed. By further evaporation of the neutral fluid on a water-bath, a gummy mass separated on the bottom of the dish. It was quite tenacious, something like rubber, soluble in cold water, but not so readily in hot. The cold water solution of the gummy mass, and the mother-liquor showed the following reactions:

1. Heated in a test-tube, the solution quickly became turbid, giving finally, if concentrated, a sticky precipitate. The turbidity, however, immediately disappeared as the fluid cooled, returning again when heat was applied and disappearing when cold. When the solution was so concentrated as to yield a heavy precipitate by heat, it naturally dissolved more slowly.
2. With chloroplatinic acid, a heavy yellow precipitate was formed, readily soluble in alcohol.
3. Heated with acetic acid there was no change, but addition of potassium ferrocyanide to the acid fluid caused a heavy precipitate.
4. Millon's reagent gave a strong reaction similar to the albumin reaction.
5. Strong potassium hydroxide gave a fine flocculent precipitate, insoluble in excess of the alkali.
6. Pure dilute nitric acid gave a heavy precipitate, only slowly soluble in excess of the acid. Heated, it yielded a clear yellow fluid and with ammonia gave the orange yellow color of the xanthoprotein reaction.
7. Pure dilute hydrochloric acid yielded a slight precipitate, soluble in large excess.
8. Saturation of the solution with ammonium sulphate gave a heavy, gummy precipitate.

The gummy mass and the original filtrate giving approximately the same reactions, the solution of the former was united with the latter, the fluid made slightly acid with acetic acid and then saturated in the cold with ammonium sulphate. The saturated fluid, on being boiled, gave a second gummy precipitate which was added to the first.

From analogy with the reactions of the proteoses, ammonium sulphate should precipitate any elastose bodies formed, leaving in solution the true peptone. The precipitates formed in the manner described were washed by being rubbed up in a mortar with hot saturated ammonium sulphate solution, and the washings added to the filtrate. A portion of the ammonium sulphate was then removed from the solution by concentration and crystallization, and the remainder by dialysis in running water. On testing the solution for peptone, however, none could be found; the biuret test failed to give any reaction, as did also phosphotungstic acid.

### *Protoelastose.*

The ammonium sulphate precipitate of elastoses, after being washed as described above, was dissolved in water, the solution made quite neutral by addition of a little sodium carbonate and then saturated with crystals of rock salt. A heavy gummy precipitate resulted, which by analogy should consist of what we may call protoelastose.

This precipitate, after being washed with saturated salt solution, was dissolved in water and reprecipitated by salt, this process being repeated three times. The final sodium chloride precipitate was dissolved in water, dialyzed until all chlorine was removed from the solution, concentrated and precipitated with 95 per cent. alcohol. Apparently, only a portion of the substance was precipitated by alcohol, so the alcoholic filtrate was evaporated and the residue carefully dried. Further examination, however, showed that both products were identical, alcohol simply precipitating a portion of the substance.

After being dried at 110° C. until of constant weight, having then the form of a brown powder, both products were analyzed with the results shown in the accompanying tables.

ANALYSIS OF PROTOELASTOSE (A1) PRECIPITATED BY ALCOHOL.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·6852 | 0·4271 | 6·92 | 1·3190 | 52·49 | ---- | ---- | ---- | ---- | ---- | -- |
| II | 0·3926 | 0·2437 | 6·89 | 0·7526 | 52·27 | ---- | ---- | ---- | ---- | ---- | -- |
| III | 0·5400 | ---- | ---- | ---- | ---- | 72·9 | 13·7 | 759·4 | 16·13 | ---- | -- |
| IV | 0·3883 | ---- | ---- | ---- | ---- | 51·9 | 15·4 | 762·9 | 15·95 | ---- | -- |
| V | 0·7048 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0259 | 3·67 |
| VI | 0·5362 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0202 | 3·76 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 54·51 | 54·28 | ---- | ---- | *54·39* |
| H | 7·19 | 7·16 | ---- | ---- | *7·17* |
| N | ---- | ---- | 16·75 | 16·56 | *16·65* |
| O | ---- | ---- | ---- | ---- | *21·79* |
| | | | | | *100·00* |

ANALYSIS OF PROTOELASTOSE (A1) FROM THE ALCOHOLIC FILTRATE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4936 | 0·3113 | 7·00 | 0·9626 | 53·18 | .... | .... | .... | .... | .... | .. |
| II | 0·4096 | 0·2592 | 7·03 | 0·8079 | 53·78 | .... | .... | .... | .... | .... | .. |
| III | 0·4588 | .... | .... | .... | .... | 63·2 | 13·4 | 759·8 | 16·49 | .... | .. |
| IV | 0·4267 | .... | .... | .... | .... | 62·5 | 21·6 | 760·8 | 17·06 | .... | .. |
| V | 0·3655 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0054 | 1·4 |
| VI | 0·5096 | .... | .... | .... | ... | .... | .... | .... | .... | 0·0075 | 1·4 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 53·96 | 54·58 | .... | .... | *54·27* |
| H | 7·11 | 7·13 | .... | .... | *7·12* |
| N | .... | .... | 16·73 | 17·31 | *17·02* |
| O | .... | .... | .... | .... | *21·59* |
| | | | | | *100·00* |

The reactions of the two preparations were identical and were as follows; both were readily soluble in cold water, but only slightly soluble in hot water; in neutral solution, heat produced a turbidity which disappeared on cooling; the concentrated mineral acids gave precipitates soluble in excess; phosphotungstic acid, picric acid, tannic acid, 30 per cent. acetic acid saturated with salt, acetic acid and potassium ferrocyanide, and alcohol all produced precipitates; mercuric chloride and mercuric nitrate gave precipitates insoluble in excess; lead acetate and cupric sulphate both failed to give any precipitate; potassium mercuric iodide produced a precipitate in a hydrochloric acid solution; sodium carbonate and sodium hydroxide gave heavy precipitates and both the biuret and xanthoprotein test gave positive results. Further, the elastose is but slightly, if at all, diffusible. Long continued concentration of an aqueous solution leads to a separation of more or less of the substance as a gummy mass.

In composition, the protoelastose formed by action of the dilute acid and heat is almost identical with that of elastin itself.

Morochowetz,* in a recent paper on the laws of digestion, states that elastin by the action of heat and water passes into a new form.

* Loc. cit.

elastose, which by further action passes into peptone. The elastose is described as a body completely soluble in water, not precipitable by mineral or acetic acids, but its solution is rendered turbid by heat. In its other reactions it resembles albumin. In composition, however, it differs decidedly from elastin and equally from our protoelastose. Morochowetz ascribes to it 55·9 per cent. carbon, 7·29 per cent. hydrogen, 16·68 per cent. nitrogen and 0·617 per cent. sulphur. As to the method employed in its preparation and purification we do not know, but as described it evidently is not akin to pure elastose.

### *Deuteroelastose.*

In the salt-saturated filtrate from the first sodium chloride precipitate of protoelastose, there was present a substance precipitable as a gummy mass, by the addition of a little 30 per cent. acetic acid saturated with salt. Past experience with other proteoses has shown that, as a rule, all of the proto body is not precipitated by saturation of its neutral solution with salt, but that there usually remains a certain amount which on addition of salt-saturated acetic acid is precipitated, together with more or less of the deutero body, and that from this filtrate pure deutero can be separated by saturation with ammonium sulphate. In the present instance, however, it appeared that the acetic acid precipitate was of quite a different nature from the sodium chloride precipitate and that if it was not pure deuteroelastose, it at least contained only a trace of the proto body. Further, saturation of the acetic acid filtrate with ammonium sulphate gave only a slight precipitate, even when the mixture was heated, showing that if the salt-saturated acetic acid had not precipitated the deuteroelastose, little could have been formed.

The gummy precipitate separated by acetic acid and salt was purified somewhat by solution in water, and reprecipitation by saturation with salt and addition of a little salt-saturated acetic acid. It was then dissolved in water, made exactly neutral to test papers, and dialyzed until all chlorine was removed from the solution. The filtered fluid was then evaporated to dryness on a water-bath, and the powdered residue dried at 110° C. until of constant weight.

The composition of the substance is shown in the accompanying table, from which it is seen that the deutero body contains a noticeably smaller percentage of carbon than protoelastose, and a correspondingly higher content of oxygen. The difference between the sodium chloride precipitate and the acetic acid precipitate, however, becomes far more marked when the reactions of the two bodies are

compared. Deuteroelastose was readily soluble both in cold and hot water, and on heating a cold saturated solution only a slight turbidity was seen which, as in the proto reaction, disappeared when the solution cooled. This turbidity was no doubt due to the presence of a small amount of protoelastose, for later on we obtained a purer deutero body having nearly all of the reactions of the present preparation in which, however, the heat reaction was absent.

ANALYSIS OF DEUTEROELASTOSE. A 1.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. | | | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | c. c. | T. ° C. | Pressure mm. | | | |
| I | 0·5164 | ---- | ---- | 0·9894 | 52·24 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·4003 | 0·2499 | 6·93 | 0·7708 | 52·51 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·4648 | 0·2924 | 6·98 | 0·8885 | 52·12 | ---- | ---- | ---- | ---- | ---- | ---- |
| IV | 0·4278 | 0·2704 | 7·02 | 0·8149 | 51·94 | ---- | ---- | ---- | ---- | ---- | ---- |
| V | 0·3624 | ---- | ---- | ---- | ---- | 49·8 | 13·8 | 759·8 | 16·43 | ---- | ---- |
| VI | 0·4818 | ---- | ---- | ---- | ---- | 65·8 | 13·9 | 759·3 | 16·30 | ---- | ---- |
| VII | 0·5370 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0105 | 1·95 |
| VIII | 0·4371 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0091 | 2·08 |

*Percentage composition of ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·30 | 53·57 | 53·19 | 52·99 | ---- | ---- | *53·26* |
| H | ---- | 7·07 | 7·13 | 7·13 | ---- | ---- | *7·12* |
| N | ---- | ---- | ---- | ---- | 16·76 | 16·64 | *16·70* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·92* |
| | | | | | | | *100·00* |

The deutero body, likewise, gave no precipitate whatever with the concentrated mineral acids, nor with acetic acid and potassium ferrocyanide. Alcohol gave no precipitate and salt-saturated 30 per cent. acetic acid, when added to an aqueous solution of the substance, also gave no precipitate.

Ferric chloride gave a slight turbidity, but no precipitate. With the other reactions of protoelastose, the deutero body showed close agreement. Pure deuteroelastose, formed later on by the action of pepsin-hydrochloric acid, gave no precipitate whatever with concentrated potassium hydroxide. This reaction, which is quite characteristic of protoelastose, was fairly distinct in the deutero body first described, which fact, coupled with the turbidity produced by heat, may be considered good evidence that the deuteroelastose was not perfectly pure.

*Digestion of elastin by pepsin-hydrochloric acid.*

An artificial gastric juice, free from both albumose and peptone, was prepared as follows; the cardiac portions from the mucous membranes of ten pigs' stomachs (820 grams) were placed in three litres of 0·4 per cent. hydrochloric acid and warmed at 40° C. for two weeks. The clear fluid filtered from the small residue of nuclein, antialbumid, etc., was then saturated with ammonium sulphate to precipitate the pepsin. This was filtered off, washed with saturated ammonium sulphate solution to remove any adherent peptone, dissolved in two litres of 0·2 per cent. hydrochloric acid and dialyzed until the sulphate was entirely removed. The resultant solution was mixed with an equal volume of 0·4 per cent. hydrochloric acid, making a pure and active pepsin-acid mixture.

In the first digestion of elastin, 150 grams of powdered elastin *A*, 1500 c.c. of the pepsin-acid mixture, and an equal volume of 0·4 per cent. hydrochloric acid, were warmed at 40° C. for 75 hours. At the end of this time the elastin was almost entirely dissolved. The acid fluid was filtered from the small residue remaining, and neutralized with potassium hydroxide, without giving any neutralization precipitate. The reactions of the fluid showed plainly the presence of elastoses similar to those formed by the action of acid. Saturation of the fluid with ammonium sulphate gave a heavy gummy precipitate, in the filtrate from which, nothing having the reactions of peptone could be discovered.

*Protoelastose.*

The ammonium sulphate precipitate was dissolved in water and the neutral fluid saturated with sodium chloride, by which a heavy gummy precipitate was formed, having all of the reactions described as characteristic of protoelastose. The product was purified by several reprecipitations with salt, then dialyzed and the solution evaporated to dryness on a water-bath, the residue powdered and dried at 110° C. until of constant weight.

The composition of the substance (protoelastose A 2) is shown in the accompanying table.

In reactions, the product agrees exactly with the protoelastose already described, being precipitated by the concentrated mineral acids, by acetic acid and potassium ferrocyanide, by 30 per cent. acetic acid and sodium chloride, by strong potassium and sodium hydroxide as well as by sodium carbonate. Its aqueous solution, likewise, shows the peculiar action towards heat, already described;

becoming turbid when heated, clear again as the solution cools. The product also gives the other reactions common to both proto- and deuteroelastose.

ANALYSIS OF PROTOELASTOSE. A 2.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4607 | 0·2880 | 6·94 | 0·9103 | 53·88 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·4952 | 0·3079 | 6·90 | 0·9763 | 53.76 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·5546 | ---- | ---- | ---- | ---- | 76·2 | 13·9 | 759·4 | 16·41 | ---- | ---- |
| IV | 0·3169 | ---- | ---- | ---- | ---- | 45·8 | 21·0 | 754·8 | 17·06 | ---- | ---- |
| V | 0·4603 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0061 | 1·32 |
| VI | 0·5303 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0073 | 1·37 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 54·58 | 54·46 | ---- | ---- | *54·52* |
| H | 7·03 | 6·99 | ---- | ---- | *7·01* |
| N | ---- | ---- | 16·64 | 17·29 | *16·96* |
| O | ---- | ---- | ---- | ---- | *21·51* |
| | | | | | *100·00* |

This body, which we prefer to call protoelastose, is apparently identical in composition and reactions with the *hemielastin* previously described by Horbaczewski,* and separated by him from a pepsin-acid digestion of elastin, by a method somewhat similar to the one employed by us.

At first glance, it would appear from Horbaczewski's method of separation, that his hemielastin would consist of a mixture of proto- and deuteroelastose, instead of being identical with the proto body. But the fact that he *strongly acidified* his digestive mixture with acetic acid, prior to saturating it with salt, explains the matter. Deuteroelastose, which is precipitable from a salt-saturated solution by a little acetic acid, is more or less soluble when excess of the acid is added, and hence by acidifying the mixture sufficiently, the deutero might remain dissolved while the proto would be precipitated by salt from an acid solution, equally as well as from a neutral fluid.

* Ueber das Verhalten des Elastins bei der Pepsinverdauung. Zeitschrift für physiologische Chemie, Band vi, p. 330.

### *Deuteroelastose.*

In the salt-saturated filtrates from protoelastose, deutero was separated as a gummy, sticky precipitate by addition of a little 30 per cent. salt-saturated acetic acid. It was purified by reprecipitation and dialysis, and the final neutral solution was evaporated to dryness and the residue dried at 110° C. until of constant weight. The amount was small and only the nitrogen was determined, 16·88 per cent.

In reactions, the body closely resembled the deutero already described; its aqueous solution giving no precipitate or turbidity whatever on the application of heat, no precipitate with alcohol, or with strong potassium hydroxide solution. It was likewise not precipitated by acetic acid and potassium ferrocyanide, by the concentrated or dilute mineral acids, nor by 30 per cent. acetic acid. In reactions, the body resembles the elastin peptone of Horbaczewski, like it being soluble in cold and warm water and dilute alcohol, and giving the same precipitations with phosphotungstic acid, picric acid, tannic acid, and with potassium mercuric iodide. It also gives the biuret and xanthoprotein reaction. It diffuses slowly.

With our present knowledge regarding peptones and proteoses, this body can hardly be considered as belonging to the former class, since it is precipitable both by ammonium sulphate, and by acetic acid when added to a salt-saturated solution; reactions not common to true peptones.

A second digestion of elastin *A* was made with the same quantities of pepsin-hydrochloric acid, and the same amount of powdered elastin, as in the first digestion. The products formed were separated in the same manner as the preceding, the elastoses being first precipitated collectively by saturation of the digestive fluid with ammonium sulphate.

On boiling the filtrate from this ammonium sulphate precipitate, a second gummy mass separated. This was collected on a cloth filter and washed with saturated ammonium sulphate solution. In the filtrate from this precipitate, no peptone could be detected. This second ammonium sulphate precipitate was purified by dialysis, etc., and the final solution evaporated to dryness and the substance dried at 110° C. Its composition is shown in the accompanying table, from which it is seen to be nearly identical with that of protoelastose. The reactions, however, indicate that it is a mixture of the two elastoses. Thus, while the concentrated mineral acids and potassium hydroxide give no precipitate, acetic acid and potassium ferrocyanide

produce a noticeable turbidity, as does also alcohol, and acetic acid and sodium chloride. Moreover, the aqueous solution becomes somewhat clouded on heating, clearing up again as the solution cools. Hence, the reactions would indicate a preponderance of deuteroelastose, while the composition points to an excess of the proto body.

ANALYSIS OF THE SECOND AMMONIUM SULPHATE PRECIPITATE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. ° C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4783 | 0·3115 | 7·23 | 0·9404 | 53·61 | --- | --- | ---- | ---- | ----- | --- |
| II | 0·3781 | 0·2433 | 7·14 | 0·7405 | 53·40 | --- | --- | ---- | ---- | ----- | --- |
| III | 0·4100 | ----- | --- | ----- | ---- | 57·7 | 14·2 | 758·0 | 16·76 | ----- | --- |
| IV | 0·3963 | ----- | --- | ----- | ---- | 57·6 | 22·6 | 753·9 | 16·72 | ----- | --- |
| V | 0·4756 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0048 | 1·00 |
| VI | 0·5016 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0054 | 1·07 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 54·14 | 53·93 | ---- | ---- | *54·03* |
| H | 7·30 | 7·22 | ---- | ---- | *7·26* |
| N | ---- | ---- | 16·92 | 16·89 | *16·90* |
| O | ---- | ---- | ---- | ---- | *22·81* |
| | | | | | *100·00* |

We must conclude, then, that this second gummy precipitate is simply a residue of the mixed elastoses not at first precipitated, perhaps from a lack of complete saturation with ammonium sulphate. Past experience, however, has shown that deuteroproteose is not as quickly or completely precipitated with ammonium sulphate as the other proteoses.

From the first ammonium sulphate precipitate, a large amount of protoelastose (A 3) was separated, having all of the reactions characteristic of this body, and the composition, when dried at 110° C., shown in the accompanying table.

A larger amount of deuteroelastose was separated from this digestion than in the preceding one, and after purification by the use of methods already described, a portion was dried at 110° C. for analysis. Its composition is shown in the accompanying table. Like the deuteroelastose formed by dilute hydrochloric acid, it contains a lower percentage of carbon than elastin or the proto body, while its content of nitrogen is higher than that of elastin. Its reactions were the same as the deutero previously obtained.

Deuteroelastose appears to be the same as the elastin peptone of Horbaczewski, as already mentioned; and the composition of the present product resembles it, in that it contains a similar percentage of carbon, but is unlike it in containing one per cent. more nitrogen.

ANALYSIS OF PROTOELASTOSE. A 3.

| No | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | T. ° C. | Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·2990 | 0·1906 | 7·07 | 0·5841 | 53·27 | --- | --- | ---- | ---- | ----- | --- |
| II | 0·4383 | 0·2750 | 6·97 | 0·8606 | 53·54 | --- | --- | ---- | ---- | ----- | --- |
| III | 0·3938 | ----- | --- | ----- | ---- | 57·1 | 19·8 | 760·8 | 16·99 | ----- | --- |
| IV | 0·3233 | ----- | --- | ----- | ---- | 47·1 | 21·6 | 757·2 | 16·89 | ----- | --- |
| V | 0·5562 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0081 | 1·45 |
| VI | 0·4866 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0079 | 1·62 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 54·08 | 54·37 | ---- | ---- | *54·27* |
| H | 7·19 | 7·08 | ---- | ---- | *7·13* |
| N | ---- | ---- | 17·26 | 17·16 | *17.21* |
| O | ---- | ---- | ---- | ---- | *21·39* |
| | | | | | *100·00* |

ANALYSIS OF DEUTEROELASTOSE. A 3.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | T. ° C. | Pressure mm. | N % | Ash found gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5067 | 0·3125 | 6·85 | 0·9681 | 52·10 | --- | --- | ---- | ---- | ----- | --- |
| II | 0·6021 | 0·3752 | 6·92 | 1·1587 | 52·47 | --- | --- | ---- | ---- | ---- | --- |
| III | 0·4550 | 0·2705 | 6·60 | 0·8685 | 52·05 | --- | --- | ---- | ---- | ---- | --- |
| IV | 0·6886 | ----- | --- | ----- | ---- | 96·3 | 13·8 | 759·4 | 16·70 | --- | --- |
| V | 0·3570 | ----- | --- | ----- | ---- | 51·4 | 22·4 | 754·0 | 16·58 | ---- | --- |
| VI | 0·6638 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0201 | 3·02 |
| VII | 0·7748 | ----- | --- | ----- | ---- | --- | --- | ---- | ---- | 0·0247 | 2·91 |

*Percentage composition of ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 53·68 | 54·07 | 53·63 | ---- | ---- | *53·79* |
| H | 7·06 | 7·13 | 6·60 | ---- | ---- | *6·99* |
| N | ---- | ---- | ---- | 17·21 | 17·31 | *17·26* |
| O | ---- | ---- | ---- | ---- | ---- | *21·96* |
| | | | | | | *100·00* |

Horbaczewski's elastin peptone is described as not giving a precipitate with acetic acid and neutral salt. Deuteroelastose, likewise, gives no precipitate with acetic acid and salt, except when the solution is saturated or nearly saturated with the salt.

Another preparation of deuteroelastose was separated from a pepsin-hydrochloric acid digestion of elastin B. Its chemical composition is shown in the accompanying table, from which it is seen to differ somewhat from deutero A, but like it has a lower content of carbon than the proto body.

ANALYSIS OF DEUTEROELASTOSE *B*.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | T. °C. | Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5919 | 0·3560 | 6·68 | 1·0934 | 50·37 | .... | .... | .... | .... | .... | .... |
| II | 0·4201 | 0·2572 | 6·80 | 0·7796 | 50·60 | .... | .... | .... | .... | .... | .... |
| III | 0·4852 | .... | .... | .... | .... | 64·9 | 14·0 | 756·8 | 15·91 | .... | .... |
| IV | 0·4719 | .... | .... | .... | .... | 66·0 | 22·4 | 754·2 | 16·11 | .... | .... |
| V | 0·7960 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0398 | 5·00 |
| VI | 0·5882 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0297 | 5·04 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 53·01 | 53·21 | .... | .... | *53·11* |
| H | 7·03 | 7·14 | .... | .... | *7·08* |
| N | .... | .... | 16·81 | 16·90 | *16·85* |
| O | .... | .... | .... | .... | *22·96* |
| | | | | | *100·00* |

*Digestion of elastin with pancreatic juice.*

A strong solution of pure trypsin was prepared, according to Kühne's method,* from 100 grams of dried ox pancreas and added to 150 grams of powdered elastin B in 2 litres of 0·5 per cent. sodium carbonate. The mixture was warmed at 40° C. for four or five days, a little thymol being added to prevent putrefaction. At the end of this time, the elastin was nearly all dissolved and the filtered fluid on being tested gave the ordinary elastose reactions. On saturation of a portion of the neutralized fluid with ammonium sulphate, a heavy gummy precipitate was obtained, and in the filtrate no trace of peptone reaction could be found.

* Kühne and Chittenden, Zeitschrift für Biologie, Band xix, p. 196.

The entire digestive fluid was therefore neutralized with dilute hydrochloric acid (no neutralization precipitate), and the protoelastose at once separated by saturation with sodium chloride. The product was purified by repeated precipitation with salt, and after dialysis, the resultant fluid was evaporated to dryness and the residue dried at 110° C. for analysis. The product contained considerable ash (7·4 per cent.), consisting mainly of calcium phosphate and sulphate, with some oxide of iron. Its composition, seen in the accompanying table, is somewhat different from the protoelastose formed by pepsin-hydrochloric acid, containing as it does a noticeably lower percentage of carbon. In its reactions, however, it resembles closely the preceding preparations. It is readily soluble in cold water and the solution when heated gives the characteristic turbidity, which disappears as the solution cools. With acetic acid and potassium ferrocyanide, it gives the usual precipitate and also with the concentrated mineral acids, the latter dissolving in an excess of the acid. Unlike the protoelastose previously described, however, it gives with cupric sulphate a precipitate soluble in excess of the copper salt, and with sodium hydroxide no precipitate. In all other respects, its reactions resemble those of the proto body formed in pepsin digestion.

ANALYSIS OF THE SODIUM CHLORIDE PRECIPITATE—TRYPSIN DIGESTION OF *B*.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4455 | 0·2635 | 6·57 | 0·8045 | 49·24 | .... | .... | .... | .... | .... | ... |
| II | 0·4650 | 0·2696 | 6·44 | 0·8360 | 49·03 | .... | .... | .... | .... | .... | ... |
| III | 0·3765 | .... | .... | .... | .... | 49·7 | 16·0 | 765·4 | 15·77 | .... | ... |
| IV | 0·6754 | .... | .... | .... | .... | 87·3 | 15·1 | 765·4 | 15·49 | .... | ... |
| V | 0·6473 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0480 | 7·41 |
| VI | 0·8286 | .... | .... | .... | .... | .... | .... | .... | .... | 0·0610 | 7·36 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 53·15 | 52·95 | .... | .... | *53·05* |
| H | 7·09 | 6·95 | .... | .... | *7·02* |
| N | .... | .... | 17·02 | 16·74 | *16·88* |
| O | .... | .... | .... | .... | *23·05* |
| | | | | | *100·00* |

In the salt-saturated filtrate from protoelastose, the addition of a little 30 per cent. acetic acid saturated with salt gave a second

gummy precipitate which was dissolved in water, the solution neutralized and dialyzed until all chlorine was removed. The solution, on evaporation, left a brownish residue, which was powdered and dried at 110° C. It was readily soluble in cold and hot water, its solution showing no turbidity whatever when heated. It likewise gave no precipitate with alcohol. Unlike the ordinary deutero-elastose, it did give noticeable precipitates with the mineral acids, soluble in excess, and also with acetic acid and potassium ferrocyanide. Lead acetate and cupric sulphate also gave precipitates, soluble in excess of the metallic salt. Sodium or potassium hydroxide failed to give any precipitate. In composition too, the product showed an approach to protoelastose formed by pepsin digestion, but unfortunately it contained over 5 per cent. of ash, and hence the quantitative results may perhaps be questionable.

ANALYSIS OF THE PRECIPITATE PRODUCED BY SALT-SATURATED ACETIC ACID—TRYPSIN DIGESTION OF *B*.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4120 | 0·2466 | 6·65 | 0·7780 | 51·49 | ---- | ---- | ---- | ---- | ---- | -- |
| II | 0·3625 | 0·2185 | 6·69 | 0·6899 | 51·89 | ---- | ---- | ---- | ---- | ---- | -- |
| III | 0·3421 | ---- | ---- | ---- | ---- | 45·6 | 14·7 | 756·5 | 15·81 | ---- | -- |
| IV | 0·3436 | ---- | ---- | ---- | ---- | 45·8 | 19·4 | 754·8 | 15·52 | ---- | -- |
| V | 0·6973 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0370 | 5·30 |
| VI | 0·5663 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0337 | 5·00 |

*Precentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 54·46 | 54·85 | ---- | ---- | *54·65* |
| H | 7·01 | 7·07 | ---- | ---- | *7·04* |
| N | ---- | ---- | 16·71 | 16·40 | *16·55* |
| O | ---- | ---- | ---- | ---- | *21·76* |
| | | | | | *100·00* |

Of anything corresponding to heteroalbumose, we have found no trace. Further, under the conditions of our experiments, no appreciable amount of true peptone was formed in any of the digestions; at least, nothing approaching a peptone in reactions was to be found in any of the digestive fluids, after saturation with ammonium sulphate. We propose, later, to attempt a study of the elastin peptone.

TABLE SHOWING COMPOSITION OF THE VARIOUS PRODUCTS ANALYZED.

| | Elastin A. | Elastin B. | Action of acid water at 100° C. | | | Action of pepsin-hydrochloric acid. | | | | | Pancreatic digestion of B. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Protoelastose A1. | | Deutero. A1. | Protoelastose. | | Deuteroelastose. | | A3. Second $(NH_4)_2SO_4$ P. P. | NaCl Precipitate. | NaCl + $CH_3COOH$ Precipitate. |
| | | | Alcohol P. P. | Filtrate. | | A2. | A3. | A3. | B. | | | |
| C | 54·24 | 54·08 | 54·39 | 54·27 | 53·26 | 54·52 | 54·27 | 53·79 | 53·11 | 54·03 | 53·05 | 54·65 |
| H | 7·27 | 7·20 | 7·17 | 7·12 | 7·12 | 7·01 | 7·13 | 6·99 | 7·08 | 7·26 | 7·02 | 7·04 |
| N | 16·70 | 16·85 | 16·65 | 17·02 | 16·70 | 16·96 | 17·21 | 17·26 | 16·85 | 16·90 | 16·88 | 16·55 |
| S | .... | 0·30 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| O | 21·79 | 21·57 | 21·79 | 21·59 | 22·92 | 21·51 | 21·39 | 21·96 | 22·96 | 21·81 | 23·05 | 21·76 |
| Ash | 0·90 | 0·31 | 3·71 | 1·47 | 2·01 | 1·34 | 1·53 | 2·96 | 5·02 | 1·03 | 7·38 | 5·45 |

using for this purpose the elastoses just described as well as elastin itself, and more vigorous digestive fluids, both peptic and tryptic.

In conclusion, then, we may say that elastin by the action of very dilute acid at 100°. C., and by the proteolytic action of pepsin-hydrochloric acid, and of trypsin yields two primary cleavage products, which bear essentially the same relation to elastin that proto- and deuteroalbumose do to albumin. Further, that protoelastose is practically identical in reactions and composition with Horbaczewski's hemielastin.

The close relationship in composition of all the bodies analyzed is shown in the accompanying table. In considering these results, however, it is well to remember that the pancreatic preparations contain an over large amount of ash, and hence the figures may not be altogether trustworthy.

## III.—The Influence of Urethan, Paraldehyde, Antipyrin, and Antifebrin on proteid metabolism. By R. H. Chittenden.

I. *The influence of urethan*;—from experiments made by *N. P. Washburn, Ph.B.*

Ethyl-urethan, $CO(NH_2)O.C_2H_5$, which has recently come into use as a hypnotic, is claimed by R. v. Jaksch* to be a sleep-producing agent, free from the usual disagreeable after effects common to most hypnotics. According to this observer, urethan does not appear to exert any poisonous action on the heart, nor to have any depressing influence on the arterial system. It is further assumed that the drug is without influence on digestion. Urethan acts mainly on the brain, the peripheral nervous system being unaffected, and according to v. Jaksch the hypnotic effect is always produced when the drug is taken in doses of 1 gram.

Smaller doses (0·25–0·5 gram) are uncertain in their action.

In view of the somewhat peculiar action of urethan as a hypnotic, we have undertaken to study the influence of the drug on the proteid metabolism of the healthy organism, in order to compare its action in this respect with that of other well known hypnotics.

Garnier,† alone, appears to have experimented in this direction. He states that, in the case of a man, a dose of 6 grams of urethan was followed by giddiness, etc., which condition, however, soon passed away. In this experiment, the excretion of urea was appreciably increased. In another experiment, the same observer found that a dose of 2 grams of urethan was also followed by an increased excretion of urea, in the case of a dog in a condition of hunger. A portion of the urethan was apparently converted into urea and excreted as such, but the greater part of the drug appeared in the urine unchanged. Garnier further states that large, nearly fatal doses, of the drug check

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* Abstract in Jahresbericht für Thierchemie, 1885, p. 70.

† Influence de l'urethane sur l'excretion des éléments azotés de l'urine. Compt. rend. soc. Biolog. 1886, p. 230.

metabolism, diminishing the quantity of urine excreted, as well as the amount of urea, uric acid, etc.

The experiments about to be described were conducted wholly upon the person of a healthy man of 66 kilos. body weight. A definite amount of food, of known composition, was taken daily and uniform habits of sleep, exercise, etc., were kept up during the whole time of the experiment, which extended over a period of six weeks. In this manner, body equilibrium was established and the daily excretions brought to a constancy of composition, as preliminary to studying the action of the drug. The daily diet was as follows:

| | | |
|---|---|---|
| 312 | grams | fresh beef, free from fat and tendons. |
| 368 | " | potatoes. |
| 227 | " | wheat bread. |
| 149 | " | boiled rice. |
| 35 | " | butter. |
| 28 | " | sugar. |
| 6 | " | salt. |
| 1200 | " | water. |

The body-weight was ascertained each morning, and the 24 hours' urine collected and analyzed each day as follows: nitrogen was determined by the Kjeldahl method;* sulphur by fusing a given volume of the urine with pure potassium hydroxide and potassium nitrate in a silver crucible, and ultimately precipitating and weighing the sulphur as barium sulphate;† phosphorus by fusion of a portion of the urine in a like manner with potassium hydroxide and nitrate, precipitation of the phosphoric acid from a nitric acid solution by molybdic solution, solution of this precipitate in ammonia and reprecipitation with magnesia mixture, as ammonio-magnesium phosphate; chlorine by ignition with potassium nitrate, and titration of the prepared solution with a standard solution of silver nitrate.

On April 19th, analysis of the urine was commenced and continued for fourteen days before the first dose of urethan was administered. The drug was then taken for five consecutive days, a total of 73 grains or 4·73 grams, after which the urethan was discontinued for seven days. A second trial was then made by giving 89 grains

* Neue methode zur Bestimmung des Stickstoffs in organischen Körpern. Zeitschrift für Analytische Chemie, xxii, 366.

† For the details of the processes used, see Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 88.

NORMAL URINE—WITHOUT URETHAN.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Nitrogen. | Sulphur. | Phosphorus | Chlorine. | Amount of urethan taken. |
|---|---|---|---|---|---|---|---|---|---|
| April | grams | | | c. c. | grams | grams | grams | grams | |
| 19 | 66400 | acid | 1027 | 950 | 17·484 | 1·210 | 1·128 | 5·000 | 0 |
| 20 | 66000 | " | 1026 | 1060 | 17·303 | 1·326 | 1·183 | 5·775 | |
| 21 | 66000 | " | 1028 | 930 | 16·707 | 1·278 | 1·166 | 4·952 | |
| 22 | 65800 | " | 1028 | 1010 | 16·891 | 1·160 | 1·141 | 6·191 | |
| 25 | 66200 | " | 1027 | 950 | 16·732 | 1·077 | 1·128 | 6·529 | |
| 26 | 66000 | " | 1023 | 1140 | 17·332 | 1·161 | 1·082 | 6·352 | |
| 27 | 65800 | " | 1023 | 1142 | ---- | 1·132 | 1·057 | 6.788 | |
| 28 | 65600 | " | 1022 | 1242 | 16·945 | 1·110 | 1·088 | 7·767 | |
| 29 | 65600 | " | 1023 | 1086 | ---- | 1·115 | 1·050 | 6·186 | |
| 30 | ---- | " | 1025 | 1132 | ---- | 1·264 | 1·195 | 7·850 | |
| May | | | | | | | | | |
| 1 | 65400 | " | 1028 | 1000 | 16·964 | 1·302 | 1·050 | 8·730 | |
| 2 | 65600 | " | 1028 | 900 | 17 392 | 1·217 | 1·010 | 5·795 | |
| 3 | 66000 | " | 1027 | 936 | 17·189 | 1·137 | 1·045 | 6·375 | |
| 4 | 65600 | " | 1026 | 1020 | 16·569 | 1·099 | 1·098 | 7·263 | |
| Average | | | 1025·8 | 1035 | 17·046 | 1·185 | 1·101 | 6·539 | |

UNDER THE INFLUENCE OF URETHAN.

| Date. | Body Weight. | Re-action. | Sp. Gr. | Total volume. | Nitro-gen. | Sul-phur. | Phos-phorus | Chlo-rine. | Amount of urethan taken. |
|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | grams | grams | grains |
| 5 | 65600 | acid | 1024 | 1188 | 16·161 | 1·163 | 1·184 | 8·459 | 5 |
| 6 | 65400 | " | 1018 | 1344 | 16·455 | 1·118 | 1·079 | 7·073 | 10 |
| 7 | .... | " | 1019 | 1200 | 15·287 | 1·118 | 1·147 | 5·572 | 16 |
| 8 | 65200 | " | 1021 | 1165 | 15·568 | 1·184 | 1·473 | 7·068 | 20 |
| 9 | 65200 | " | 1029 | 900 | 16·384 | 1·201 | 1·197 | 6·241 | 22 |
| 10 | 65200 | " | 1028 | 930 | 17·704 | 1·214 | 1·056 | 5·528 | 0 |
| 11 | 64800 | " | 1028 | 980 | 17·644 | 1·404 | 1·138 | 6·371 | |
| 12 | 64600 | " | 1028 | 970 | 18·706 | .... | .... | .... | |
| 13 | 64400 | " | 1021 | 1496 | 20·411 | 1·358 | 1·177 | 7·410 | |
| 14 | .... | " | 1021 | 1254 | 16·258 | 1·306 | 1·146 | 5·512 | |
| 15 | 64400 | " | 1020 | 1336 | 18·836 | 1·264 | 1·073 | 7·776 | |
| 16 | 64200 | " | 1023 | 1182 | 18·680 | 1·272 | 1·090 | 6·367 | |
| 17 | 64200 | " | 1020 | 1412 | 18·485 | 1·419 | 1·130 | 8·130 | 20 |
| 18 | 63600 | " | 1016 | 1622 | 17·522 | 1·166 | 0·997 | 7·331 | 30 |
| 19 | 63200 | " | 1022 | 1130 | 16·597 | 1·265 | 0·977 | 7·707 | 39 |
| 20 | 63000 | " | 1026 | 1000 | 17·860 | 1·241 | 1·062 | 6·191 | 0 |
| 21 | .... | " | 1026 | 950 | 18·397 | 1·269 | 1·093 | 5·558 | |
| 22 | 63600 | " | 1021 | 1300 | 21·690 | 1·431 | 1·170 | 7·405 | |
| 23 | 63600 | " | 1023 | 1116 | 20·192 | 1·442 | 1·156 | 5·873 | |
| 24 | 63400 | " | 1019 | 1424 | 20·340 | 1·332 | 1·150 | 7·142 | |

or 5·76 grams of urethan in three consecutive days, followed by a period of six days in which the drug was not taken.

The tables, giving the amounts in grams for the 24 hours' urine of the several elements determined, show plainly that urethan has a decided action on the metabolism of the body.

On examining the results in detail, it is to be seen that urethan has a very decided diuretic action, most noticeable on the second day the drug was taken. In fact, this may be called the initial action of the drug, since in both trials the amount of water excreted, after the first increase, rapidly diminished as the dose of urethan was increased, and indeed, the volume remained far below the average amount for two or three days after the drug had been discontinued, or until its elimination from the system was fairly complete, when the volume of fluid quickly rose to normal.

The excretion of nitrogen is at once affected by urethan, even a dose of five or ten grains bringing the nitrogen noticeably below the normal amount. In both series, the excretion of nitrogen was greatly diminished. On discontinuing the drug, the nitrogen excreted rapidly increased in amount, and on the third or fourth day after its discontinuance, the daily excretion of nitrogen passed considerably above the normal.

As regards the excretion of phosphorus, it would appear from the experiments that the administration of small doses of urethan gives rise to an increased excretion of this element, as seen from the results obtained on May 5–9th. With larger doses of the drug, however, the excretion of phosphorus is diminished, as seen from the results of May 17–19th. As the excretion of sulphur runs parallel with the excretion of nitrogen, both coming from the metabolism of proteid matter, it follows that urethan when taken in small quantities must exert an inhibitory influence on proteid metabolism, while it stimulates the decomposition of certain phosphorized matters. In larger doses, the inhibitory action of the drug on proteid metabolism is still more pronounced, while at the same time the excretion of phosphorus is also retarded.

In no case was any hypnotic action noticeable.

II. *The influence of Paraldehyde;*—from experiments made by *J. E. Dockdorff, Ph.B.*

In the following experiment, a full-blooded coach dog of 25 kilos weight was employed. The animal was confined in a suitable cage, lined with galvanized iron and furnished with a bottom of wire netting, under which was a funnel-shaped tray, the whole so arranged as to allow all of the fluid excreta to pass into a collecting bottle underneath.

The animal was fed daily on a weighed diet consisting of dessicated beef, soda crackers and water. The beef was prepared by removing as thoroughly as possible all fat, fasciæ, tendons, etc., passing it through a sausage cutter and then drying it at a temperature below 60° C., until it had lost 75 per cent. of its weight. The dried and sampled beef was then preserved in tightly stoppered jars until needed. The crackers were ordinary soda crackers, containing about 0·7 per cent. of nitrogen. The daily rations consisted of 60 grams of crackers and 125 grams of the dessicated beef, soaked in 600 c. c. of water. This diet was commenced sometime before the urine was collected, and was continued throughout the experiment. Ultimately, the 24 hours' urine was analyzed each day, according to the methods described in the preceding experiment. Owing to irregularity of urination, and the difficulty of using a catheter, the quantity of urine obtained each day was necessarily quite variable, hence the composition of the normal urine was determined daily for three weeks, so that a sufficiently large number of results might be obtained to yield an accurate average for the normal period. Paraldehyde was then administered in gelatin capsules, about six hours after the dog had been fed, so that the drug might not interfere with digestion. Its administration was continued for eighteen days.

The results, expressed in grams for each 24 hours' urine, are shown in the accompanying tables.

NORMAL URINE. WITHOUT PARALDEHYDE.

| Date. | Total Volume. | Sp. Gr. | Reaction. | Nitrogen. | Sulphur. | Phosphorus. | Amount of Paraldehyde. |
|---|---|---|---|---|---|---|---|
| April | c. c. | | | grams | grams | grams | |
| 21 | 395 | 1036 | acid. | 12·540 | 0·883 | 0·862 | 0 |
| 22 | 840 | 1025 | " | 19·315 | 1·529 | 1·288 | |
| 23 | 660 | 1031 | " | 18·276 | 1·176 | 1·442 | |
| 24 | 490 | 1035 | " | 14·622 | 1·038 | 1·096 | |
| 25 | 610 | 1036 | " | 21·050 | 1·388 | 1·340 | |
| 26 | 410 | 1036 | " | 13·568 | ---- | ---- | |
| 27 | 550 | 1037 | " | 18·550 | 1·396 | 1·202 | |
| 28 | 415 | 1036 | " | 13·580 | 1·322 | 0·730 | |
| 29 | 420 | 1037 | " | 14·916 | 0·940 | 0·934 | |
| 30 | 630 | 1038 | " | 18·071 | 1·394 | 1·386 | |
| May | | | | | | | |
| 1 | 490 | 1040 | " | 18·415 | 1·172 | 1.214 | |
| 2 | 390 | 1038 | " | 12·896 | 0·826 | 0·806 | |
| 3 | 630 | 1038 | " | 23·425 | 1·499 | 1·410 | |
| 4 | 525 | 1036 | " | 17·244 | 1·235 | 1·098 | |
| 5 | 430 | 1035 | " | 13·849 | 1·138 | 0·862 | |
| 6 | 375 | 1035 | " | 11·672 | 0·810 | 0·784 | |
| 7 | 740 | 1032 | " | 23·191 | 1·648 | 1·538 | |
| 8 | 465 | 1031 | " | 13·682 | 1·085 | 0·898 | |
| 9 | 545 | 1032 | " | 16·239 | 1·340 | 1·166 | |
| 10 | 535 | 1032 | " | 15·756 | 1·119 | 1·134 | |
| 11 | 495 | 1032 | " | 14·406 | 1·145 | 0·904 | |
| Av'age | 526 | 1035 | acid. | 16·440 | 1·204 | 1·105 | |

WITH PARALDEHYDE $(C_2H_4O)_3$.

| Date. | Total Volume. | Sp. Gr. | Reaction. | Nitrogen. | Sulphur. | Phosphorus. | Amount of Paraldehyde. |
|---|---|---|---|---|---|---|---|
| May | c. c. | | | grams | grams | grams | grams |
| 12 | 540 | 1031 | acid. | 15·032 | 1·346 | 1·044 | 0·424 |
| 13 | ... | Urine | lost. | ------ | ---- | ---- | 0·740 |
| 14 | 540 | 1028 | acid. | 13·624 | 1·000 | 0·776 | 0·715 |
| 15 | 810 | 1024 | " | 6·589 | 0·587 | 0·442 | 0·794 |
| 16 | 810 | 1027 | " | 20·191 | 1·593 | 1·339 | 0·755 |
| 17 | 460 | 1031 | " | 12·793 | 0·874 | 0·932 | 0·784 |
| 18 | 690 | 1033 | " | 21·091 | 1·444 | 1·296 | 0·702 |
| 19 | 525 | 1033 | " | 16·863 | 1·214 | 1·106 | 1·071 |
| 20 | 505 | 1032 | " | 14·523 | 1·016 | 0·978 | 1·536 |
| 21 | 735 | 1029 | " | 20·844 | 1·475 | 1·292 | 1·499 |
| 22 | 545 | 1031 | " | 16·756 | 1·122 | 0·462 | 1·811 |
| 23 | 555 | 1031 | " | 17·063 | 1·195 | 1·620 | 2·268 |
| 24 | 580 | 1032 | " | 18·202 | 1·245 | 1·080 | 2·675 |
| 25 | 360 | 1030 | " | 9·935 | 0·734 | 0·590 | 3·111 |
| 26 | 800 | 1033 | " | 25·695 | 1·924 | 1·476 | 3·632 |
| 27 | 460 | 1030 | " | 12·992 | 0·935 | 0·766 | 4·063 |
| 28 | 585 | 1033 | " | 19·174 | 1·368 | 0·990 | 4·958 |
| 29 | 695 | 1032 | " | 21·756 | 1·391 | 1·342 | 5·941 |
| Av'age | 570 | 1030 | acid. | 16·060 | 1·203 | 1·031 | 37·479 |

WITHOUT PARALDEHYDE.

| Date. | Total Volume. | Sp. Gr. | Reaction. | Nitrogen. | Sulphur. | Phosphorus. | Amount of Paraldehyde. |
|---|---|---|---|---|---|---|---|
| May | c. c. | | | grams | grams | grams | |
| 30 | 610 | 1028 | acid. | 17·672 | 1·309 | 0·992 | 0 |
| 31 | 570 | 1031 | " | 17·080 | 1·311 | 1·154 | |
| June 1 | 440 | 1028 | " | 12·201 | 0·765 | 0·780 | |
| 2 | 645 | 1031 | " | 19·550 | 1·376 | 1·299 | |
| 3 | 640 | 1030 | " | 17·798 | 1·237 | 1·608 | |
| 4 | 445 | 1026 | " | 11·112 | 0·979 | .... | |
| Av'age | 558 | 1029 | acid. | 15·902 | 1·163 | 1·166 | |

Throughout the experiment, the dog appeared perfectly well, and at no time showed any symptoms of nausea. Neither was there any special hypnotic action noticeable.

The average of the results shows plainly that the drug has little, if any, action on proteid metabolism. Under the influence of the paraldehyde there was a slight increase in the amount of water excreted. Bokai * has stated that the urinary secretion is slightly increased by paraldehyde. In our experiment, however, the diuretic action is not great. As regards the excretion of nitrogen, there is a slight diminution to be seen in the paraldehyde period. There is also a corresponding decrease in the excretion of phosphorus. The three periods, however, show such close agreement in results, it is obvious that, under the conditions of this experiment, paraldehyde has not exerted any special influence on proteid metabolism.

According to the experiments of Quinquad and A. Hénocque,† paraldehyde causes a diminution of body temperature, and at the same time a very noticeable falling off in the excretion of carbonic acid; thus, according to one of Quinquad's experiments, a dog after receiving by hypodermic injection 8 c. c. of paraldehyde gave off 5·5 grams of carbonic acid, while it expired during the same time, three-fourths of an hour after the injection, only 1·96 grams of carbonic acid.

* Ueber die physiologische Wirkung des Paraldehyds. Centralblatt für die medicinische Wissenschaften. 1887, p. 412.

† Abstract in Jahresbericht für Thierchemie. 1884, p. 374.

III. *Influence of Antipyrin*;—from experiments made by *H. F. Adams, Ph.B.*

Previous experiments* with antipyrin, made in this laboratory, have shown that this drug, when introduced into the stomach of healthy rabbits, has little, if any, noticeable influence on the excretion of carbonic acid or on the body temperature, except in the case of toxic doses. Coppola,† likewise, found that the subcutaneous introduction of 0·1 to 0·3 gram of antipyrin in the case of dogs led to a reduction of body temperature of only 0·25 to 0·6 of a degree, while 0·3–0·4 gram of the drug was without any noticeable influence upon the excretion of urea. In fever patients, F. Müller,‡ however, had previously noticed a diminution in the excretion of nitrogen under the influence of antipyrin, while in the case of healthy men the excretion was affected but very slightly, if at all. Jacubowitsch,§ likewise, had noticed in experiments on healthy and fevered children that the use of antipyrin led to a diminution both in the quantity and specific gravity of the urine, and also a diminution in the quantity of uric acid, phosphoric acid, sulphuric acid and of chlorides. L. Riess,‖ by carefully conducted experiments on nine typhus fever patients, found as a principal result that antipyrin in doses up to 12 grams per day diminished considerably the excretion of nitrogen, the diminution ranging in six series of experiments from 2·5 to 24·7 per cent. Umbach,¶ likewise, has studied the influence of antipyrin on the excretion of nitrogen, both on a dog and on himself, and he found that with a definite diet the excretion of nitrogen sank, under the influence of 4 grams of antipyrin, in two days about 2 grams, equal to 4 grams of urea. The uric acid excretion, however, was not materially affected.

In spite of these manifold experiments, we have deemed the matter of sufficient importance to warrant further study, especially with a view to the action of the drug on the metamorphism of nitrogenous matter in the *healthy* organism. The experiments were therefore tried upon a healthy man with a body weight of 77 kilograms, and

* Chittenden and Cummins. Studies, vol. ii., p. 231.

† Abstract in Jahresbericht für Thierchemie. 1885, p. 98.

‡ Abstract in Jahresbericht für Thierchemie. 1884, p. 242.

§ Abstract in Jahresbericht für Thierchemie. 1885, p. 444.

‖ Archiv für experim. Pathol. u. Pharm. xxii, 127; also Abstract in Jahresbericht für Thierchemie. 1886, p. 417. Ueber stickstoffausscheidung bei antipyretischer Fieberbehandlung.

¶ Ueber den Einfluss der Antipyrins auf die stickstoffausscheidung. Abstract in Jahresbericht für Thierchemie. 1886, p. 418.

under definite conditions of diet, exercise, etc. Nitrogenous equilibrium was established prior to the experiment, and the following daily diet was strictly adhered to throughout the entire period.

| | | |
|---|---|---|
| 386 | grams | meat (beef). |
| 840 | " | potatoes. |
| 227 | " | wheat bread. |
| 168 | " | oat meal (steamed). |
| 28 | " | sugar. |
| 42 | " | butter. |
| 120 | " | milk. |
| 1040 | " | water. |

In this experiment, nitrogenous metabolism was measured by determining the urea and uric acid contained in the daily excretion, instead of determining the total nitrogen. This was done in order to ascertain whether the drug has any special action on the excretion of uric acid. Urea was determined by Liebig's method, as modified by Pflüger.* Chlorine was previously determined by fusion with potassium nitrate and titration with silver nitrate in the usual manner, and then removed from the solution to be tested for urea, by a standard silver solution. Uric acid was determined by Salkowski's method, and phosphoric acid by titration with a standard uranium solution.

The accompanying tables give the results of the daily analyses. From these it is evident that under the conditions of this experiment, antipyrin has a decided inhibitory action on the proteid metabolism of the healthy human organism, as shown by the diminished excretion of urea and uric acid when the drug is taken. Antipyrin also tends to diminish the volume of the urinary secretion, this action being very marked in the second series, where comparatively large amounts of antipyrin were administered. As regards the excretion of phosphoric acid and of chlorine, nothing definite can be said. The more important changes produced by the antipyrin are shown in the following table of average daily results.

| | Urea. grams. | Uric acid. gram. | Total $P_2O_5$. grams. | Volume. c. c. | Sp.Gr. |
|---|---|---|---|---|---|
| Normal period | 41·806 | 0·586 | 3·185 | 951 | 1028 |
| First antipyrin period | 38·375 | 0·556 | 3·026 | 848 | 1029 |
| First after period | 42·080 | 0·575 | 2·929 | 929 | 1028 |
| Second antipyrin period | 40·854 | 0·472 | 2·941 | 822 | 1031 |
| Second after period | 44·220 | 0·537 | 2·923 | 957 | 1028 |

* Pflüger's Archiv für Physiologie, vol. xxi, p. 248.

NORMAL URINE.—WITHOUT ANTIPYRIN.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Chlorine. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. | Amount of Antipyrin taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| April. | grams | | | c. c. | grams | grams | gram | gram | grams | |
| 24 | 77000 | acid | 1030 | 840 | 4·660 | 3·264 | 0·492 | 0·543 | 42·359 | 0 |
| 25 | 77200 | " | 1028 | 970 | 6·557 | 3·126 | 0·524 | 0·595 | 42·120 | |
| 26 | 77200 | " | 1027 | 950 | 3·820 | 3·236 | 0·499 | 0·570 | 41·427 | |
| 27 | 77200 | " | 1025 | 1085 | 5·407 | 3·043 | 0·509 | .... | 41·026 | |
| 28 | 77000 | " | 1027 | 1040 | 6·113 | 3·341 | 0·541 | 0·702 | 42·768 | |
| 29 | 77000 | " | 1026 | 1090 | 5·781 | 2·957 | 0·561 | 0·651 | 41·616 | |
| 30 | 76900 | " | 1027 | 1000 | 6·006 | 3·284 | 0·601 | 0·636 | 41·492 | |
| May | | | | | | | | | | |
| 1 | 76800 | " | 1029 | 970 | 6·756 | 3·166 | 0·586 | 0·560 | 41·861 | |
| 2 | 76800 | " | 1030 | 870 | 4·113 | 3·400 | 0·483 | 0·542 | 42·141 | |
| 3 | 76800 | " | 1030 | 860 | 4.117 | 3·238 | 0·495 | 0·571 | 41 913 | |
| 4 | 76600 | " | 1031 | 790 | 3·279 | 2·980 | 0·483 | 0·491 | 41·143 | |
| Average | | | 1028 | 951 | 5·146 | 3·185 | 0·525 | 0·586 | 41·806 | |

UNDER THE INFLUENCE OF ANTIPYRIN.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Chlorine. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid. | Urea. | Amount of Antipyrin taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | gram | gram | grams | grains |
| 5 | 76600 | acid | 1031 | 800 | 3·016 | 3·288 | 0·599 | 0·501 | 40·995 | 30 |
| 6 | 76600 | " | 1031 | 780 | 3·688 | 3·118 | 0·596 | 0·548 | 38·391 | 30 |
| 7 | 76700 | " | 1027 | 1050 | 5·009 | 2·559 | 0·535 | 0·625 | 38·060 | 30 |
| 8 | 76800 | " | 1029 | 800 | 4·089 | 3·059 | 0·610 | 0·548 | 37·168 | 45 |
| 9 | 76600 | " | 1030 | 810 | 3·985 | 3·106 | 0·608 | 0·557 | 37·260 | 54 |
| Average | | | 1029 | 848 | 3·975 | 3·026 | 0·588 | 0·556 | 3 ·375 | |
| 10 | 76800 | " | 1028 | 900 | 4·658 | 2·652 | 0·550 | 0·607 | 39·744 | 0 |
| 11 | 76400 | " | 1026 | 890 | 3·867 | 2·541 | 0·567 | 0·602 | 42·895 | |
| 12 | 76400 | " | 1028 | 910 | 4·203 | 2·821 | 0·555 | 0·602 | 41·860 | |
| 13 | 75800 | " | 1028 | 1000 | 4·281 | 3·009 | 0·535 | 0·498 | 43·080 | |
| 14 | 75800 | " | 1028 | 915 | 3·625 | 3·265 | 0·589 | 0·582 | 42·510 | |
| 15 | 75800 | " | 1028 | 910 | 4·535 | 3·202 | 0·598 | 0·642 | 42·110 | |
| 16 | 75600 | " | 1028 | 980 | 4·759 | 3·011 | 0·514 | 0·495 | 42·012 | |
| Average | | | 1028 | 929 | 4·275 | 2·929 | 0·558 | 0·575 | 42·030 | |

UNDER THE INFLUENCE OF ANTIPYRIN—*Continued.*

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Chlorine. | Total $P_2O_5$. | $P_2O_5$ with Ca and Mg. | Uric acid | Urea | Amount of Antipyrin taken. |
|---|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | gram | gram | grams | grains |
| 17 | 75400 | acid | 1030 | 880 | 4·442 | 3·286 | 0·542 | 0·534 | 41·694 | 40 |
| 18 | 75600 | " | 1030 | 880 | 4·048 | 3·335 | 0·627 | 0·564 | 42·404 | 50 |
| 19 | 75800 | " | 1032 | 790 | 4·341 | 3·052 | 0·635 | 0·406 | 41·143 | 60 |
| 20 | 74600 | " | 1032 | 760 | 4·278 | 2·748 | 0·588 | 0·385 | 39·227 | 60 |
| 21 | ..... | " | 1032 | 800 | 4·805 | 2·284 | 0·574 | 0·472 | 39·804 | 60 |
| Average | | | 1031 | 822 | 4·382 | 2·941 | 0·593 | 0·472 | 40·854 | |
| 22 | 75400 | " | 1030 | 910 | 4·477 | 2·652 | 0·590 | 0·621 | 44·076 | 0 |
| 23 | 74800 | " | 1028 | 990 | 3·795 | 2·632 | 0·500 | 0·516 | 45·114 | |
| 24 | 74800 | " | 1027 | 970 | 4·834 | 2·945 | 0·495 | 0·533 | 43·751 | |
| 25 | 74800 | " | 1027 | 965 | 3·576 | 3·196 | 0·532 | 0·514 | 45·490 | |
| 26 | 74600 | " | 1029 | 950 | 3·885 | 3·190 | 0·520 | 0·504 | 42·673 | |
| Average | | | 1028 | 957 | 4·113 | 2·923 | 0·527 | 0·587 | 44·220 | |

Since this work was finished we have seen an interesting paper by Dr. Kumagawa* on the action of certain antipyretics on proteid metabolism, in which is given the results of an experiment with antipyrin on the excretion of nitrogen and uric acid, in the case of a dog of 26 kilos. weight in a condition of nitrogenous equilibrium.

In this experiment, Dr. Kumagawa found that even large doses of antipyrin (51 grams in 16 days) produced no change whatever in the excretion of nitrogen (determined by the Kjeldahl method), but that there was a very noticeable increase in the excretion of uric acid (determined by Salkowski's method), amounting on an average to 65 per cent. above the normal excretion. These results stand in direct opposition to what we have found with somewhat smaller doses, in experimenting on the human organism. Whether the explanation of this difference is to be found in the different nature of the two organisms experimented with we cannot now say, but we hope at a later date to explain this apparently divergent action.

IV. *The influence of antifebrin;*—from experiments made by *H. C. Taylor, Ph.B.*

Antifebrin or acetanilide, which has recently come into use as an antipyretic, as a nervine and antiseptic, has been the subject of many clinical observations but has not as yet, so far as we know, been experimented with to ascertain its influence on proteid metabolism. We have endeavored, therefore, to ascertain the influence of this new antipyretic on the nutrition of the healthy human organism, believing that such results may possibly be of greater value than those obtained by experimenting on animals. At the same time it is to be borne in mind, that an antipyretic especially may produce an effect upon the healthy organism quite different from that which the same doses would produce on an organism rendered perhaps more susceptible by disease, as in fever. The experiment was therefore conducted upon the person of a young man of 64 kilos. body weight, brought into a condition of nitrogenous equilibrium and maintained throughout the experiment upon a weighed diet of known composition. For reasons already given, the excretion of nitrogenous matter was measured by determining in the 24 hours' urine the amount of urea and uric acid, using the methods employed in one of the preceding experiments. Sulphur, phosphorus and chlorine were also

*Ueber die Wirkung einiger antipyretischer Mittel auf den Eiweissumsatz im Organismus. Virchow's Archiv, Band cxiii, p. 192.

determined each day by methods already indicated. After nitrogenous equilibrium had been established, and the urine analyzed for ten consecutive days, antifebrin was administered daily in divided doses, at a time not to interfere with digestion, for a period of nine days. The daily dose was gradually increased until at last there was a slight approach to cyanosis. In all, 13·3 grams or 205 grains of the antipyretic were taken. No disagreeable symptoms were experienced, but there was a very noticeable lividity of countenance with a decided blueness of the lips, and a slight darkening of the skin near the cheek bones.

The initial daily dose was 0·4 of a gram or a little over 6 grains, and was rapidly increased to 2·6 grams or 40 grains per day, given in three doses. There are, to be sure, many cases recorded where apparently smaller doses have led to serious results, but careful watching failed to show any symptoms whatever even suggestive of any disagreeable action on the digestive system, the circulation or respiration. Weinstein, indeed, has said that persons not suffering from fever may take antifebrin for weeks together without any effect on the circulation, while according to Herczel the long-continued administration of antifebrin, thirty to forty-five grains daily for six weeks, may lead to what he terms aniline anaemia with solution and decomposition of the hæmoglobin of the blood. In fact, the latter observer considers that aniline is set free from the acetanilide and that the decomposition of the blood-coloring matter is due to this cause. Whether this is the cause of the cyanosis so often spoken of in connection with this drug is uncertain.

In the second antifebrin period, 13·9 grams or 214 grains of the drug were taken in seven days, accompanied at the close with the same approach to cyanosis as before.

Examination of the analytical results shows plainly that under the conditions of this experiment the excretion of urea is not very greatly affected. There is, however, in both antifebrin periods a slight increase, indicating increased proteid metabolism under the influence of the drug. This increased excretion of urea is more apparent in the individual results than in the average of the series. Thus, in the normal urine it is to be noticed that the daily excretion of urea never exceeded 34·5 grams, while in the first antifebrin period, on the days when the largest doses of acetanilide were taken, the excretion of urea amounted to 35–37 grams, and in the after period quickly fell to about 33 grams per day. The same peculiarity is also noticeable, to a less extent, in the second antifebrin period.

NORMAL URINE—WITHOUT ANTIFEBRIN.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Urea. | Uric acid. | Sulphur. | Phosphorus. | Chlorine. | Amount of Antifebrin. |
|---|---|---|---|---|---|---|---|---|---|---|
| April | grams | | | c. c. | grams | gram | grams | grams | grams | |
| 23 | 64200 | acid | 1025 | 790 | 33·331 | .... | 1·380 | 0·966 | 2·967 | 0 |
| 24 | 64200 | " | 1016 | 1280 | 33·280 | 0·749 | 1·154 | 0·864 | 4·404 | |
| 25 | 64000 | " | 1017 | 1200 | 33·486 | 0·708 | 1·207 | 1·227 | 3·603 | |
| 26 | 64000 | " | 1014 | 1340 | 33·947 | 0·739 | 1·254 | 0·859 | 3·520 | |
| 27 | 64000 | " | 1018 | 1030 | 33·058 | 0·753 | 1·220 | 1·041 | 3·221 | |
| 28 | 64000 | " | 1017 | 1150 | 34·502 | 0·779 | 1·296 | 0·881 | 4·027 | |
| 29 | 63800 | " | 1015 | 1250 | 32·619 | 0·723 | 1·222 | 0·862 | 4·377 | |
| 30 | 64200 | " | 1023 | 880 | 33·440 | 0·741 | 1·289 | 0.842 | 4·073 | |
| May | | | | | | | | | | |
| 1 | 64000 | " | 1013 | 1590 | 34·370 | 0·694 | 1·350 | 0·821 | 6·366 | |
| 2 | 64000 | " | 1016 | 1250 | 33·334 | 0·777 | 1·124 | 1·122 | 4·078 | |
| Average | | | 1017 | 1176 | 33·536 | 0·740 | 1·249 | 0·948 | 4·063 | |

FIRST ANTIFEBRIN PERIOD.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Urea. | Uric acid | Sulphur. | Phosphorus. | Chlorine. | Amount of Antifebrin. |
|---|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | grams | grams | grams | grams |
| 3 | 64000 | acid | 1015 | 1400 | 33·190 | 0·671 | 1·241 | 0·932 | 4·743 | 0·4 |
| 4 | 64000 | " | 1013 | 1630 | 34·155 | 0·762 | 1·313 | 0·910 | 5·505 | 0·7 |
| 5 | 64000 | " | 1017 | 1190 | 29·920 | 0·611 | 1·096 | 0·510 | 4·912 | 0·9 |
| 6 | 64000 | " | 1014 | 1770 | 35·063 | 0·670 | 1·380 | 1·167 | 6·533 | 1·2 |
| 7 | 64000 | " | 1018 | 1190 | 29·843 | 0·538 | 1·241 | 0·992 | 4·153 | 1·5 |
| 8 | 64000 | " | 1017 | 1300 | 33·882 | 0·651 | 1·358 | 1·012 | 0·520 | 2·4 |
| 9 | 63800 | " | 1017 | 1230 | 35·172 | 0·572 | 1·294 | 0·948 | 4·386 | 2·6 |
| 10 | 63800 | " | 1017 | 1280 | 35·996 | 0·639 | 1·299 | 0·846 | 3·843 | 2·6 |
| 11 | 63800 | " | 1015 | 1470 | 37·011 | 0·596 | 1·318 | 1·102 | 4·782 | 1·0 |
| Average | | | 1016 | 1384 | 33.804 | 0·634 | 1·282 | 0·935 | 4·375 | |

FIRST PERIOD AFTER ANTIFEBRIN.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Urea. | Uric acid. | Sulphur. | Phosphorus. | Chlorine. | Amount of Antifebrin. |
|---|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | grams | grams | grams | |
| 12 | 63800 | acid | 1017 | 1210 | 32·965 | 0·690 | 1·372 | 0·858 | 4·314 | 0 |
| 13 | 63800 | " | 1018 | 1800 | 33·596 | 0·705 | 1·374 | 0·962 | 5·405 | |
| 14 | 63600 | " | 1016 | 1400 | 33·596 | 0·650 | 1·412 | 0·965 | 4·292 | |
| 15 | 63600 | " | 1015 | 1560 | 36·245 | 0·715 | 1·214 | 1·099 | 4·465 | |
| 16 | 63600 | " | 1013 | 1830 | 34·799 | 0·785 | 1·489 | 1·089 | 5·380 | |
| 17 | 63400 | " | 1016 | 1370 | 33·437 | 0·755 | 1·443 | 0·883 | 4·484 | |
| 18 | 63400 | " | 1016 | 1400 | 33·904 | 0·755 | 1·356 | 0·878 | 5·904 | |
| 19 | 63500 | " | 1018 | 1710 | 32·736 | 0·640 | 1·588 | 0·969 | 5·472 | |
| 20 | 63600 | " | 1016 | 1500 | 32·377 | 0·645 | 1·376 | 0·980 | 6·043 | |
| Average | | | 1015 | 1531 | 33·739 | 0·704 | 1·404 | 0·967 | 5·084 | |

SECOND ANTIFEBRIN PERIOD.

| Date. | Body weight. | Reaction. | Sp. Gr. | Total volume. | Urea. | Uric acid. | Sulphur. | Phosphorus. | Chlorine. | Amount of Antifebrin. |
|---|---|---|---|---|---|---|---|---|---|---|
| May | grams | | | c. c. | grams | grams | grams | grams | grams | grams |
| 21 | 63800 | acid | 1017 | 1280 | 34·027 | 0·437 | 1·360 | 0·959 | 5·814 | 1·0 |
| 22 | 63800 | " | 1016 | 1340 | 33·678 | 0·460 | 1·312 | 0·841 | 5·814 | 2·0 |
| 23 | 63800 | " | 1019 | 1270 | 33·882 | 0·592 | 1·272 | 0·860 | 5·995 | 2·2 |
| 24 | 63800 | " | 1018 | 1340 | 33·968 | 0·570 | 1·235 | 0·876 | 7·001 | 2·5 |
| 25 | 63800 | " | 1018 | 1420 | 34·579 | 0·595 | 1·432 | 0·874 | 8·302 | 2·5 |
| 26 | 63800 | " | 1022 | 1220 | 32·990 | 0·626 | 1·249 | 0·996 | 6·295 | 2·5 |
| 27 | 63600 | " | 1018 | 1510 | 34·765 | 0·721 | 1·399 | 0·864 | 7·510 | 1·2 |
| Average | | | 1018 | 1340 | 33·983 | 0·571 | 1·323 | 0·896 | 6·604 | |
| 28 | 63600 | " | 1014 | 1800 | 33·792 | 0·596 | 1·492 | 1·017 | 8·158 | 0 |
| 29 | 63600 | " | 1022 | 1000 | 33·201 | 0·639 | 1·375 | 0·785 | 5·917 | |
| 30 | 63600 | " | 1025 | 920 | 32·880 | 0·681 | .... | 0·876 | 5·279 | |
| Average | | | 1020 | 1240 | 33·291 | 0·639 | 1·428 | 0·892 | 6·451 | |

We must conclude, therefore, that acetanilide tends to increase somewhat the excretion of urea, but that with such doses as we have employed the increase in proteid metabolism cannot be great. This is further indicated by the lack of any corresponding change in the excretion of sulphur.

The excretion of phosphorus is also unaffected by antifebrin.

On the excretion of uric acid, however, our results indicate a special inhibitory influence. This is quite apparent both in the averages of the different series and in the individual results, and, if correct, would appear to be the most marked characteristic of antifebrin, so far as its influence on proteid metabolism is concerned. Various observers have stated that antifebrin acts as a diuretic, others that it decreases the secretion of water, and while doubtless both results have been seen to follow its administration in diseased conditions of the system, our experiment on a healthy man gives no evidence of any action of this kind.

Since the foregoing was written we have seen the results of Kumagawa's* experiment with antifebrin on a dog, from which he concludes that acetanilide taken in small doses (2–3 grams per day) does not give rise to any appreciable increase in the decomposition of proteid matter, but that larger doses (4–5 grams per day) cause a very marked increase, as indicated by the increased excretion of nitrogen. Such doses are, however, as Kumagawa himself admits, too large, especially when given to a moderate sized dog, to have the results of any practical value. Lépine,† too, experimenting on a hungry dog, thought he found an increase in the excretion of nitrogen after giving two doses of one and two grams of antifebrin. Further, Ademski‡ is quoted as considering that urea is increased, but the total quantity of nitrogen decreased by antifebrin. Bokai, that the amount of nitrogen is diminished and Berezooski that the urea decreases with the fall of temperature. Whether these latter views are founded on actual experiments or are mere conjecture I do not know.

According to Jaffe and Hilbert,§ rabbits fed upon antifebrin excrete it mainly as paramidophenol-sulphuric acid, and Kumagawa has likewise found, in the case of a dog, that neither acetanilide nor aniline appear in the urine, but that the antifebrin is excreted mainly as paramidophenol united to sulphuric acid.

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* Virchow's Archiv. Band cxiii, p. 171.

† See Salkowski's Bemerkung in Virchow's Archiv, Band cxiii, p. 394.

‡ See Report on Antifebrin in the Therapeutic Gazette, vol. xii, p. 571.

§ Zeitschrift für physiologische chemie, xii, p. 307.

## IV.—The influence of several new therapeutic agents on amylolytic and proteolytic action. By R. H. Chittenden and C. W. Stewart, Ph.B.

In view of the pronounced action of a number of newly discovered therapeutic agents on metabolism, we have deemed it of importance to widen our knowledge regarding their physiological action by attempting a study of their behavior towards the amylolytic and proteolytic ferments, with the hope of gaining some insight into their influence on normal digestion.

The methods employed were similar to those used in previous experiments of this kind,* in which the action of varying percentages of the drug were determined quantitatively.

### *Influence on amylolytic action.*

As amylolytic ferment, human mixed saliva was employed, filtered and carefully neutralized, and then diluted with distilled water in the proportion of 1 to 5. The experiments were made in series, in which one digestion of each series served as a control for comparison. The volume of each digestive mixture was 100 c. c., in which was present 1 gram of perfectly neutral potato starch previously boiled with a portion of the water, 10 c. c. of diluted neutral saliva and a given quantity of the substance to be experimented with. The mixtures were warmed at 40° C. for thirty minutes, after which further action of the ferment was stopped by heating the solution to boiling. The extent of amylolytic action was then ascertained, by determining in one-fourth of the solution the amount of reducing substances by Allihn's gravimetric method.† For the sake of convenience, the total amount of reducing substance was calculated as dextrose, from which in turn was calculated the percentage of starch converted.

### *Antipyrin.*

With this new antipyretic, several series of experiments with small percentages were made which show clearly that the substance is

* Studies from the Laboratory of Physiological Chemistry, Yale University, vols. i and ii.

† Zeitschrift für analytische chemie, xxii, p. 448.

without any appreciable influence. When present in larger percentages, the drug was found to have a slight inhibitory influence on amylolytic action, as the following series of experiments show :

| Per cent. of Antipyrin. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3446 gram. | 31·01 per cent. | 100·0 |
| 0·5 | 0·3446 | 31·01 | 100·0 |
| 1·0 | 0·3424 | 30·82 | 99·4 |
| 3·0 | 0·3278 | 29·50 | 95·1 |
| 5·0 | 0·3112 | 28·01 | 93·6 |

Coppola,* in studying the physiological action of antipyrin, found that three per cent. of the substance did not hinder the inversion of cane sugar by yeast, but did prevent alcoholic fermentation. Further, that the same percentage hindered slightly the action of malt diastase on starch, and had a decided inhibitory influence on the alkaline fermentation of urine.

### *Antifebrin.*

Owing to the comparative insolubility of antifebrin or acetanilide in water, large percentages could not be employed. Such as were used, however, clearly show that this antipyretic has little influence on amylolytic action.

| Per cent. of Antifebrin. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3604 gram. | 32·44 per cent. | 100·0 |
| 0·10 | 0·3600 | 32·40 | 99·9 |
| 0·25 | 0·3638 | 32·70 | 100·8 |
| 0·50 | 0·3550 | 32·00 | 98·6 |
| 0 | 0·3382 | 30·44 | 100·0 |
| 2·0 | 0 3298 | 29·68 | 97·5 |

In this connection, it is interesting to notice that Kumagawa * has found antifebrin to have a strong antiseptic action on the putrefactive processes of the intestinal canal, and Van Seer has observed that milk does not undergo fermentation if saturated with it, and also that it will prevent albumin becoming putrid.†

### *Urethan.*

With ethyl-urethan, two series of experiments were tried, with the following results :

* Jahresbericht für Thierchemie. 1885, p. 98.

† Virchow's Archiv, Band cxiii, p. 184.

‡ See The Therapeutic Gazette, vol. xii, p. 566.

| Per cent. of Urethan. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3382 gram. | 30·44 per cent. | 100·0 |
| 0·5 | 0·3550 | 31·95 | 104·6 |
| 0 | 0·3402 | 30·62 | 100·0 |
| 2·0 | 0·3446 | 31·01 | 101·3 |
| 3·0 | 0·3352 | 30·17 | 98·5 |
| 5·0 | 0·3268 | 29·40 | 96·0 |

Here, there is noticeable, with the smaller percentages, a slight stimulation of amylolytic action, but it is not sufficiently large to be very marked.

### *Paraldehyde.*

This substance shows very strong inhibitory action, even 0·5 per cent. diminishing the amount of starch converted by 30 per cent. It must be remembered, however, that the aldehyde is only slightly soluble in water, and that it is more or less volatile. The experiments were conducted in small flasks and the aldehyde kept more or less emulsionized with the aqueous solution by shaking the mixtures, but obviously the percentages given can only approximately represent the amount actually taking part in the reaction.

Following are the results of two series of experiments:

| Per cent. of Paraldehyde. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3554 gram. | 31·98 per cent. | 100·0 |
| 0·1 | 0·3534 | 31·81 | 99·5 |
| 0·2 | 0·3528 | 31·75 | 99·3 |
| 0·5 | 0·2468 | 22·21 | 69·5 |
| 1·0 | 0·1066 | 9·59 | 30·0 |
| 0 | 0·3554 | 31·98 | 100·0 |
| 0·8 | 0·1392 | 12·52 | 39·1 |
| 1·0 | 0·0948 | 8·53 | 26·6 |
| 1·5 | 0·0620 | 5·58 | 14·3 |
| 2·0 | 0·0446 | 4·01 | 12·5 |

### *Thallin Sulphate.*

This salt has a more marked influence on the amylolytic action of saliva than any of the preceding substances. Very small percentages have a noticeable stimulating action, while the presence of 0·2 per cent. of the salt almost entirely stops the action of the ferment. The results of the following two series show one or two small points of difference, but in the main they point to the same general action.

| Per cent. of Thallin Sulphate. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3534 gram. | 31·81 per cent. | 100·0 |
| 0·025 | 0·3648 | 32·83 | 103·2 |
| 0·050 | 0·3810 | 34·36 | 108·0 |
| 0·100 | 0·3540 | 31·86 | 100·1 |
| 0·200 | 0·0690 | 6·21 | 19·5 |
| 0·300 | trace. | | |
| 0 | 0·3652 | 32·86 | 100·0 |
| 0·025 | 0·3798 | 34·18 | 104·0 |
| 0·050 | 0·3872 | 34·89 | 106·1 |
| 0·080 | 0·3812 | 34·81 | 105·9 |
| 0·10 | 0·3148 | 28·33 | 86·2 |

### *Caffein and Thein.*

These two closely related alkaloids were found to have only a slight action on the amylolytic ferment, producing when present in considerable amount a slight diminution in the amount of starch converted. With two per cent. of the alkaloids, the following results were obtained:

| Per cent. of alkaloid. | Total amount of reducing substances. | Starch converted. | Relative amylolytic action. |
|---|---|---|---|
| 0 | 0·3620 gram. | 32·57 per cent. | 100·0 |
| 2·0 Caffein. | 0·3342 | 30·08 | 92·4 |
| 0 | 0·3664 | 32·97 | 100·0 |
| 0·2 Thein. | 0·3440 | 30·96 | 93·9 |

### *Influence on proteolytic action.*

The influence of the above therapeutic agents on the proteolytic action of pepsin-hydrochloric acid was determined, as in preceding work of this kind,* by ascertaining the amount of fibrin digested or dissolved in a given time, by a definite volume of standard, artificial gastric juice, in the presence of varying amounts of the substances to be tested. The gastric juice was made by dissolving 10 c. c. of a glycerin extract of pepsin in one litre of 0·2 per cent. hydrochloric acid. The volume of each digestive mixture was 50 c. c., composed of 25 c. c. of the above mentioned artificial gastric juice, and 25 c. c. of 0·2 per cent. hydrochloric acid containing the desired amounts of the substances to be tested. The proteid material consisted of purified fibrin, coarsely powdered and dried at 110° C. One gram of fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for a certain length of time, usually two hours,

* Studies from the Laboratory of Physiological Chemistry of Yale University, vols. i and ii.

after which further proteolytic action was stopped by heating the mixtures to boiling. The undissolved residues were then collected on dried, weighed filters, washed thoroughly with boiling water, and finally dried at 110° C. until of constant weight. The amount of fibrin dissolved is taken as a measure of proteolytic action.

Following are the results obtained with the various substances tested:

### *Antipyrin.*

| Per cent. of Antipyrin. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·507 gram. | 49·3 per cent. | 100·0 |
| 0·2 | 0·522 | 47·8 | 96·9 |
| 0·5 | 0·573 | 42·7 | 86·6 |
| 1·0 | 0·688 | 31·2 | 63·3 |
| | Longer time at 40° C. | | |
| 0 | 0·143 | 85·7 | 100·0 |
| 0·5 | 0·213 | 78·7 | 91·8 |
| 3·0 | 0·964 | 3·6 | 4·2 |
| 5·0 | 1·008 | 0 | 0 |

### *Antifebrin.*

| Per cent. of Antifebrin. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·129 gram. | 87·1 per cent. | 100·0 |
| 0·1 | 0·145 | 85·5 | 98·1 |
| 0·2 | 0·166 | 83·4 | 95·8 |
| 0·5 | 0·212 | 78·8 | 90·5 |
| 1·5 | 0·371 | 62·9 | 72·2 |

### *Urethan.*

| Per cent. of Urethan. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·148 gram. | 85·2 per cent. | 100·0 |
| 0·2 | 0·138 | 86·2 | 101·2 |
| 1·0 | 0·171 | 82·9 | 97·2 |
| 3·0 | 0·224 | 77·6 | 90·9 |
| 5·0 | 0·240 | 76·0 | 89·4 |

### *Paraldehyde.*

| Per cent. of Paraldehyde. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·263 gram. | 73·7 per cent. | 100·0 |
| 0·05 | 0·219 | 78·1 | 105·9 |
| 0·10 | 0·246 | 75·4 | 102·3 |
| 0·30 | 0·255 | 74·5 | 101·1 |
| 1·00 | 0·264 | 73·6 | 99·9 |
| 2·00 | 0·269 | 73·1 | 99·2 |

*Thallin sulphate.*

| Per cent. of Thallin sulphate. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·379 gram. | 62·1 per cent. | 100·0 |
| 0·05 | 0·243 | 75·7 | 121·9 |
| 0·10 | 0·327 | 67·3 | 108·4 |
| 0·30 | 0·395 | 60·5 | 97·4 |

*Thein and Caffein.*

| Per cent. of alkaloid. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·105 gram. | 89·5 per cent. | 100·0 |
| 1·0 Thein. | 0·252 | 74·8 | 84·7 |
| 4·0 " | 0·179 | 82·1 | 91·8 |
| 4·0 Caffein. | 0·584 | 41·6 | 46·5 |

These results show that antipyrin has a decided inhibitory influence on the action of the proteolytic ferment, and that when present to the extent of 3·0 per cent. it practically stops all digestive action. Antifebrin has also an inhibitory action, perhaps equal to that of antipyrin, but owing to its greater insolubility in acid fluids large percentages of the substance could not be tested.

Urethan has only a slight retarding action, even when present to the extent of 5 per cent.

Paraldehyde and thallin sulphate both show a very decided stimulating action when small fractions of one per cent. are present, the latter, particularly, causing a much larger amount of fibrin to be digested than in the control experiment.

Thein and caffein both show an inhibitory influence on the ferment, that of caffein being much greater than that of thein.

On the proteolytic action of trypsin in an alkaline solution, two substances only were tested, antifebrin and paraldehyde. The experiments were conducted in the same manner as with pepsin-hydrochloric acid, except that a solution of trypsin in 0·3 per cent. sodium carbonate was employed in place of the pepsin-acid, and the mixture warmed for a longer time at 40° C. Following are the results obtained, showing a much more pronounced inhibitory action on this ferment than on pepsin.

| Per cent. of substance. | Undigested residue. | Fibrin digested. | Relative proteolytic action. |
|---|---|---|---|
| 0 | 0·159 gram. | 84·1 per cent. | 100·0 |
| 0·2 antifebrin. | 0·368 | 63·2 | 75·1 |
| 1·0 | 0·741 | 25·9 | 30·8 |
| 0·2 paraldehyde. | 0·368 | 63·2 | 75·1 |
| 2·0 | 0·959 | 4·1 | 4·9 |

## V.—Caseoses, Casein Dyspeptone, and Casein Peptone. By R. H. Chittenden.

In a previous paper on "Casein and its Primary Cleavage Products,"* the writer expressed the intention of continuing the study of the caseoses formed in pepsin digestion, and also of studying pure casein peptone and the so-called casein dyspeptone. In the fulfillment of this intention, experiments upon these subjects have been carried on in this laboratory during the past two years, with the aid of several co-workers, and the results are here presented collectively.

### I.—*Casein Dyspeptone;*—from experiments by *L. A. Conner, Ph.B.*, and *C. A. Tuttle, Ph.B.*

When casein is exposed to the action of pepsin-hydrochloric acid at 40° C., or even at ordinary temperatures, it is decomposed, as is well known, into soluble caseoses and peptone. In every such digestion, however, there always remains a certain amount of an insoluble pasty, grayish white substance, which apparently is not susceptible to the further action of gastric juice, no matter how long continued. This insoluble substance, which is noticeable to a greater or less extent in the pepsin digestion of all proteids, but particularly so with casein, and which received from Meissner the name of dyspeptone, was examined somewhat carefully by Lubavin in 1870,† who described some of its properties. He considered this casein dyspeptone as a mixture of two distinct bodies, separable from each other by the action of sodium carbonate. Substance *A*, that portion of the dyspeptone soluble in sodium carbonate, was described by Lubavin as a body containing 4·6 per cent. of phosphorus, 13·3 per cent. of nitrogen, 48·5 per cent. of carbon and no sulphur, corresponding to the formula $C_{27}H_{47}N_6PO_{11}$. Substance *B*, insoluble in sodium carbonate, was slowly soluble in sodium hydroxide, contained sulphur, but only a small trace of phosphorus and evidently contained more or less

* Chittenden and Painter. Studies from Laboratory of Physiological Chemistry Yale University, vol. ii., p. 156.

† Hoppe-Seyler. Med. Chem. Untersuchungen, p. 463.

admixture of fat. Judging from the description, substance *A* must have been an acid compound of the body studied, mixed with more or less undigested casein; while substance *B* was doubtless a mixture of fat and the body *A*. However this may be, our results show conclusively that no body having the formula ascribed by Lubavin to his substance *A*, can be separated from the undigestible residue of casein in pepsin-hydrochloric acid.

In all of the experiments to be described, the casein employed was freshly prepared from skim milk by precipitating the greatly diluted fluid with dilute acetic acid, washing thoroughly with water, redissolving the precipitate in water containing a trace of ammonia and reprecipitating, repeating this operation three or four times.

In subjecting casein to the action of artificial gastric juice the conditions were varied more or less in the individual experiments, so that if the so-called dyspeptone be a mixture of two or more substances, the varying conditions under which the digestions were made might so change the nature of the mixture, that on analysis, it would become apparent.

### *Digestion A.*

The casein from five gallons of milk was placed in four litres of 0·4 per cent. hydrochloric acid and warmed to 40° C. To this was added 200 c. c. of a dialyzed pepsin solution, prepared from a glycerin extract of the ferment, and the mixture kept at 40–45° C. for forty-eight hours. At the end of this time there was still a comparatively large mass of gelatinous matter undissolved, composed in part, no doubt, of swollen casein. The entire mixture was then diluted considerably with water and treated with dilute alkali to near neutralization, leaving the fluid, however, distinctly acid. The undigested matter was then filtered off and washed thoroughly with water. This partial neutralization of the digestive mixture was found necessary, owing to the extreme slowness with which the acid fluid filtered. The undigested matter was again warmed at 40° C. for forty-eight hours, with four litres of a much more vigorous pepsin mixture containing 0·4 per cent. hydrochloric acid. The residue still undissolved was filtered off and washed with water. The acid filtrate gave no precipitate whatever on neutralization. A third time the undigested matter was warmed at 40° C. for sixty hours, with four litres of a still stronger artificial gastric juice. The quantity of insoluble matter did not appear to be diminished at all by this third treatment with pepsin and acid. The substance was thereupon filtered from the acid

fluid, and washed with water until the washings gave no reaction for chlorides. It was then treated in the cold with one litre of one per cent. sodium carbonate, in which it appeared to dissolve completely. On filtration there appeared a small whitish residue, which on treatment with ether, dissolved in great part, thus showing its fatty nature. There remained, however, a very small residue of a heavy, brownish substance too small in quantity to be considered other than as an impurity. On adding dilute hydrochloric acid to the alkaline fluid, no precipitate was obtained until the fluid was made distinctly acid, when the dyspeptone was thrown down as a heavy flocculent precipitate. In the filtrate, the biuret and Millon's test showed only a faint trace of an albuminous body. The precipitate of dyspeptone was washed with water until the washings gave no reaction with silver nitrate, after which it was dissolved in one per cent. sodium carbonate, the fluid made exactly neutral with dilute hydrochloric acid, thymolized, and then dialyzed in running water until all chloride was removed from the fluid.

The neutral fluid of dyspeptone so obtained, was concentrated to a thick syrup on the water-bath, and then while still warm, it was treated with 95 per cent. alcohol and a little absolute alcohol. A moderately heavy precipitate of dyspeptone resulted, but apparently not all of the substance was precipitated. On standing for forty-eight hours, the fluid was found in a thick, gelatinous condition. The coagulum was insoluble in 95 per cent. alcohol, but readily and completely soluble in water. It was therefore washed thoroughly with alcohol, allowed to stand under absolute alcohol for several days, then treated with cold ether, after which it was dried, powdered and placed in a fat extractor and extracted with boiling ether as long as any fatty matter was dissolved, a process which took several days. About nine grams of the pure, dry substance were obtained.

A portion was then dried at 110° C. until of constant weight, for analysis. Its composition is shown in the accompanying table.

The methods of analysis employed were the same as those previously described. Phosphorus was determined by fusing the substance in a silver crucible with potassium hydroxide and potassium nitrate, acidifying the mixture with nitric acid, evaporating to dryness, dissolving the residue in water acidified with nitric acid and precipitation of the phosphoric acid, first with molybdic solution, and lastly with magnesia mixture and final weighing of the phosphorus as magnesium pyrophosphate.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | $Mg_2P_2O_7$ from the Ash. gram. | P of Ash. % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | P after deducting P of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·2389 | 0·1325 | 6·04 | 0·3872 | 44·20 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·3058 | 0·1695 | 6·16 | 0·4949 | 44·13 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0.4003 | ------ | --- | ------ | ---- | 44·9 | 19·4 | 754·4 | 13·05 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·4581 | ------ | --- | ------ | ---- | 51·0 | 17·8 | 760·0 | 13·14 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·3834 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0523 | 13·64 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VI | 0·4092 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0561 | 13·70 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VII | 0·5884 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0253 | 0·59 | ---- | --- | ---- | --- | ----- |
| VIII | 0·5227 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0243 | 0·63 | ---- | --- | ---- | --- | ----- |
| IX | 0·4092 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0379 | 2·58 | ---- | --- | ----- |
| X | 0·5884 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | 0·0537 | 2·54 | 0 |
| XI | 0.5227 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | 0·0501 | 2·67 | 0·09 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·18 | 51·12 | ---- | ---- | ---- | ---- | *51·15* |
| H | 7·13 | 7·24 | ---- | ---- | ---- | ---- | *7·18* |
| N | ---- | ---- | 15·12 | 15·20 | ---- | ---- | *15·16* |
| S | ---- | ---- | ---- | ---- | 0·69 | 0·73 | *0·71* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | *0* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *25·80* |
| | | | | | | | *100·00* |

It is to be seen from the table of analytical results that the total phosphorus is exactly equal to the phosphorus of the ash. The ash, as examination showed, was composed almost entirely of calcium phosphate with a trace of iron. There was no calcium sulphate. Taking the percentage of phosphorus at the highest figure, viz: 2·67, and calculating it to calcium phosphate $Ca_3(Po_4)_2$, it would be equal to 13·3 per cent. of calcium phosphate, or within 0·3 per cent. of the ash found. Hence, it would appear that the phosphorus present in the substance probably existed there wholly as calcium phosphate.

### *Digestion B.*

A quantity of pure casein, equal in amount to that used in digestion *A*, was warmed at 40° C. with seven litres of 0·4 per cent. hydrochloric acid, to which a quantity of purified and vigorous pepsin solution was added. After being kept at 40° C. for fifty hours, two litres more of 0·4 per cent. hydrochloric acid, together with some pepsin solution, were added and the mixture warmed at 40° C. for two days more, after which it was diluted with water and the undigested residue allowed to settle out. The supernatant fluid was syphoned off, the residue washed by decantation and then again treated at 40° C. with five litres of an active pepsin-hydrochloric acid solution for four days. The residue still undigested was filtered off, washed with water, dissolved in one per cent. sodium carbonate solution and the alkaline fluid filtered from the small amount of undissolved matter. From this fluid, the dyspeptone was precipitated by hydrochloric acid, the acid compound washed thoroughly with water, after which it was warmed at 40° C. for forty-eight hours with 1200 c. c. of 0·2 per cent. hydrochloric acid, and 50 c. c. of a strong pepsin solution. The undigested residue, after being thoroughly washed, was dissolved in 800 c. c. of one per cent. sodium carbonate, the solution exactly neutralized with hydrochloric acid and dialyzed until chlorides were entirely removed. The clear aqueous solution was evaporated to a syrup and the dyspeptone precipitated with alcohol, after which it was treated exactly as preparation *A*. 9·5 grams of pure, dried substance were obtained.

For analysis, the dyspeptone was dried at 110° C. until of constant weight. The analytical results are shown in the accompanying table.

The ash which was larger than in the first preparation, contained no sulphate whatever, but was composed in great part of calcium phosphate.

DYSPEPTONE B.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | $Mg_2P_2O_7$ from the Ash. gram. | P of Ash. % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | P after deducting P of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4220 | 0·2385 | 6·28 | 0·6680 | 43·16 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·2814 | 0·1582 | 6·25 | 0·4465 | 43·27 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·4918 | ------ | --- | ------ | ---- | 55·1 | 20·2 | 754·7 | 13·02 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·3221 | ------ | --- | ------ | ---- | 35·4 | 20·1 | 758·9 | 12·84 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·4145 | ------ | --- | ------ | ---- | 47·0 | 19·4 | 758·1 | 13·26 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| VI | 0·5141 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0791 | 15·38 | ---- | --- | ---- | --- | --- | --- | ----- |
| VII | 0·4893 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0756 | 15·45 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VIII | 0·5304 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0223 | 0·58 | ---- | --- | ---- | --- | ----- |
| IX | 0·5040 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0228 | 0·62 | ---- | --- | ---- | --- | ----- |
| X | 0·4893 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0484 | 2·76 | ---- | --- | ----- |
| XI | 0·6304 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | 0·0633 | 2·80 | 0·04 |
| XII | 0·6056 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | 0·0586 | 2·72 | 0 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 51·02 | 51·13 | ---- | ---- | ---- | ---- | ---- | *51·07* |
| H | 7·42 | 7·40 | ---- | ---- | ---- | ---- | ---- | *7·41* |
| N | ---- | ---- | 15·40 | 15·23 | 15·62 | ---- | ---- | *15·41* |
| S | ---- | ---- | ---- | ---- | ---- | 0·69 | 0·73 | *0·71* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *0* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | *25·40* |
| | | | | | | | | *100·00* |

### *Digestions C and D.*

These digestions were conducted in much the same manner as the preceding. In *C*, the casein was first subjected to the action of four litres of vigorous, but purified, artificial gastric juice containing 0·4 per cent. acid, for four consecutive days. The undigested residue was then filtered off, washed, and again treated with a vigorous pepsin-acid mixture for three days longer, in both cases at 40° C. The residue still undigested was washed thoroughly with water and then dissolved in one per cent. sodium carbonate, after which it was treated exactly as preparation A.

In *D*, the casein was warmed for three days with six litres of a similar pepsin-acid mixture, when the undissolved residue, after being filtered and thoroughly washed, was dissolved in diluted sodium carbonate and reprecipitated by dilute hydrochloric acid. Then, as in *B*, the washed precipitate was redigested with a vigorous gastric juice for several days and the residue again dissolved, after thorough washing, in one per cent. sodium carbonate and treated exactly as the preceding preparation.

For analysis, both products were freed entirely from fat, and ultimately dried at 110° C., until of constant weight. Their composition is shown in the accompanying tables.

Three other distinct preparations of dyspeptone were made in manner similar to the preceding, except that in all, larger quantities of pepsin-hydrochloric acid were employed and the mixtures warmed for a longer time at 40° C. Thus in digestion *E*, 1562 grams of moist casein were warmed with 9·5 litres of 0·4 per cent. hydrochloric acid and pepsin for two days, and the undigested residue again treated at 40° C. with 3 litres of a like pepsin-acid for seven days, and finally treated a third time with pepsin and acid for four days, before solution in sodium carbonate, etc. Likewise in digestion *G*, 2000 grams of moist casein were warmed at 40° C. with 11 litres of 0·4 per cent. acid and pepsin for 21 days and the residue warmed again at 40° C. for several days, with a fresh pepsin-acid mixture. Ultimately, all of the three products were treated as previously described, and finally dried at 110° C. prior to analysis. The analytical results are shown in the accompanying tables.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T.°C. | N found. Pressure m.m. | N % | Ash found. gram. | Ash % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | $Mg_2P_2O_7$ from the ash. gram. | P of ash % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | P after deducting P of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3603 | 0·2068 | 6·38 | 0·5946 | 45·03 | --- | --- | ---- | --- | ---- | --- | ---- | --- | --- | --- | ---- | --- | ----- |
| II | 0·2849 | 0·1341 | 6·34 | 0·3857 | 44·80 | --- | --- | ---- | --- | ---- | --- | ---- | --- | --- | --- | ---- | --- | ----- |
| III | 0·6983 | ---- | ---- | ---- | ---- | 78·8 | 18·4 | 759·5 | 13·28 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·4780 | ---- | ---- | ---- | ---- | 54·9 | 18·8 | 756·8 | 13·44 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·5470 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0680 | 12·43 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VI | 0·5655 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0703 | 12·43 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VII | 0·5733 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0245 | 0·58 | ---- | --- | ---- | --- | ----- |
| VIII | 0·5908 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0262 | 0·60 | ---- | --- | ---- | --- | ----- |
| IX | 0·5655 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0469 | 2·31 | ---- | --- | ----- |
| X | 0·5733 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0508 | 2·47 | 0·16 |
| XI | 0·5908 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0524 | 2·47 | 0·16 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·40 | 51·18 | ---- | ---- | ---- | ---- | *51·29* |
| H | 7·28 | 7·24 | ---- | ---- | ---- | ---- | *7·26* |
| N | ---- | ---- | 15·16 | 15·31 | ---- | ---- | *15·23* |
| S | ---- | ---- | ---- | ---- | 0·67 | 0·69 | *0·68* |
| P | ---- | ---- | ---- | ---- | ---- | ---- | *0* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *25·54* |
| | | | | | | | *100·00* |

## DYSPEPTONE D.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T.°C. | N found. Pressure m.m. | N % | Ash found. gram. | Ash % | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | $Mg_2P_2O_7$ from the ash. gram. | P of ash % | $Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram. | P % | P after deducting P of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·2070 | 0·1200 | 6·44 | 0·3390 | 44·66 | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·2158 | 0·1236 | 6·38 | 0·3510 | 44·37 | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·4238 | ---- | ---- | ---- | ---- | 48·0 | 18·4 | 757·8 | 13·29 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·2711 | ---- | ---- | ---- | ---- | 30·9 | 20·1 | 757·6 | 13·29 | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·2070 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0294 | 14·20 | --- | --- | ---- | --- | ---- | --- | ----- |
| VI | 0·2158 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0306 | 14·18 | ---- | --- | ---- | --- | ---- | --- | ----- |
| VII | 0·6436 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0302 | 0·64 | ---- | --- | ---- | --- | ----- |
| VIII | 0·5613 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0279 | 0·68 | ---- | --- | ---- | --- | ----- |
| IX | 0·4228 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0386 | 2·55 | ---- | --- | ----- |
| X | 0·6436 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0554 | 2·40 | 0 |
| XI | 0·5613 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0508 | 2·52 | 0 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 51·97 | 51·68 | ---- | ---- | ---- | ---- | *51·82* |
| H | 7·47 | 7·41 | ---- | ---- | ---- | ---- | *7·44* |
| N | ---- | ---- | 15·48 | 15·48 | --- | ---- | *15·48* |
| S | --- | | | -- | 0·76 | 0·80 | *0·78* |
| P | -- | - | -- | - | -- | --- | *0* |
| O | - | | | | - | | *24·48* |

*Dyspeptone E.*

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·7272 | 0·3758 | 5·74 | 1·1528 | 43·23 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·3746 | 0·1927 | 5·71 | 0·5967 | 43·17 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·3177 | ---- | ---- | ---- | ---- | 34·06 | 13·0 | 759·6 | 12·84 | ---- | ---- |
| IV | 0·5570 | ---- | ---- | ---- | ---- | 58·33 | 12·2 | 760·3 | 12·61 | ---- | ---- |
| V | 0·3319 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0496 | 14·94 |
| VI | 0·3330 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0499 | 14·98 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 50·84 | 50·75 | ---- | ---- | *50·80* |
| H | 6·75 | 6·72 | ---- | ---- | *6·73* |
| N | ---- | ---- | 15·10 | 15·15 | *15·12* |

*Dyspeptone F.*

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·5375 | 0·2837 | 5·86 | 0·8772 | 44·50 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·5686 | 0·3039 | 5·93 | 0·9317 | 44·68 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·4649 | ---- | ---- | ---- | ---- | 50·3 | 12·4 | 764·0 | 13·11 | ---- | ---- |
| IV | 0·4100 | ---- | ---- | ---- | ---- | 44·7 | 13·0 | 765·5 | 13·16 | ---- | ---- |
| V | 0·3938 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0536 | 13·61 |
| VI | 0·4347 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0590 | 13·59 |

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 51·50 | 51·70 | ---- | ---- | *51·60* |
| H | 6·78 | 6·87 | ---- | ---- | *6·83* |
| N | ---- | ---- | 15·17 | 15·21 | *15·19* |

*Dyspeptone G.*

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·7545 | 0·3915 | 5·76 | 1·2148 | 43·90 | ---- | ---- | ---- | ---- | ---- | ---- |
| II | 0·4094 | 0·2137 | 5·79 | 0·6604 | 43·98 | ---- | ---- | ---- | ---- | ---- | ---- |
| III | 0·6268 | ---- | ---- | ---- | ---- | 70·6 | 13·8 | 760·5 | 13·56 | ---- | ---- |
| IV | 0·7803 | ---- | ---- | ---- | ---- | 88·8 | 14·0 | 760·3 | 13·61 | ---- | ---- |
| V | 0·4356 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0552 | 12·66 |
| VI | 0·5654 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0·0722 | 12·76 |

*Precentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 50·35 | 50·44 | ---- | ---- | *50·39* |
| H | 6·61 | 6·65 | ---- | ---- | *6·63* |
| N | ---- | ---- | 15·55 | 15·61 | *15·58* |

TABLE SHOWING THE AVERAGE COMPOSITION OF THE SEVERAL DYSPEPTONES.

| | A. | B. | C. | D. | E. | F. | G. | Casein.* |
|---|---|---|---|---|---|---|---|---|
| C | 51·15 | 51·07 | 51·29 | 51·82 | 50·80 | 51·60 | 50·39 | 53·30 |
| H | 7·18 | 7·41 | 7·26 | 7·44 | 6·73 | 6·83 | 6·63 | 7·07 |
| N | 15·16 | 15·41 | 15·23 | 15·48 | 15·12 | 15·19 | 15·58 | 15·91 |
| S | 0·71 | 0·71 | 0·68 | 0·78 | .... | .... | .... | 0·92 |
| P | 0 | 0 | 0 | 0 | .... | .... | .... | 0·87 |
| O | 25·80 | 25·40 | 25·54 | 24·48 | .... | .... | .... | 22·06 |
| Ash | 13·67 | 15·41 | 12·43 | 14·19 | 14·96 | 13·60 | 12·71 | 0·98 |

It is evident from the more complete analyses of the first four products, that the dyspeptone as prepared by us contains essentially the same percentage of sulphur as the original casein; further, that instead of being a phosphorized compound, it apparently contains no phosphorus whatever, other than that combined with calcium. Very noticeable, is the large percentage of ash in all of the preparations. This we were not able to materially reduce by any process of purification, and as the ash of the original casein, like that of the dyspeptone, was composed almost wholly of calcium phosphate, it would appear as if all of the phosphate from the mother substance had attached itself to the dyspeptone.

In their content of carbon, all of the seven preparations show a very close agreement, while they differ from casein by containing two per cent. less carbon. The nitrogen of the dyspeptone is, likewise, a little less than that of casein, while the individual preparations show throughout a very close agreement in their content of this element. In composition, therefore, all of the seven preparations, although representing considerable variation in the method of production, show a sufficiently close agreement to indicate their identity. Compared with casein, the lower percentage of carbon would point to their production by hydration, and it would appear from the analytical data that the so-called casein dyspeptone, formed by gastric digestion, is a mixture of calcium phosphate with a hydration product of casein.

* Chittenden and Painter. Studies from Laboratory, vol. ii. p. 173.

the hydrochloric acid compound of which is insoluble in water and dilute acid. The dyspeptone itself is quite readily soluble in cold water, the solution remaining unchanged on boiling.

Following are some of the reactions of dyspeptone.

Addition of acetic acid to an aqueous solution of the substance produces a heavy white precipitate insoluble in moderate excess, but partially soluble in a large excess of the acid. On heating the strongly acid fluid, the precipitate dissolves completely and the fluid remains clear on cooling. Addition of potassium ferrocyanide to the clear acid fluid gives only a slight turbidity.

Dilute hydrochloric and sulphuric acid both give a heavy white precipitate, insoluble in slight excess of acid, but entirely soluble in a large excess on application of heat. Even 0·2 per cent. hydrochloric acid precipitates the dyspeptone completely.

Dilute nitric acid, likewise, precipitates the dyspeptone, but the precipitate is far more soluble in excess of the acid. On warming the acid solution, it quickly turns yellow, and with ammonia gives the orange yellow color of the xanthoprotein reaction.

Cupric sulphate and potassium hydroxide give the violet color of the biuret reaction.

Cupric sulphate and ferric chloride both give heavy precipitates, insoluble in excess.

Potassium hydroxide and lead acetate give, on boiling, a distinct reaction for sulphur.

Mercuric chloride, added in small quantity to a cold aqueous solution of the dyspeptone, gives no precipitate, but when added in excess and the mixture is heated, a heavy white precipitate is formed, insoluble on cooling.

The dyspeptone is precipitated by saturation of its aqueous solution with ammonium sulphate, but not by sodium chloride, even on heating. Addition of acetic acid, however, to the salt-saturated fluid gives the usual precipitate of dyspeptone.

### *Casein antialbumid.*

On heating casein with sulphuric acid and water at 100° C., it is decomposed, as is well known, into soluble products and an insoluble antialbumid. We have found, however, that the antialbumid prepared in this manner is quite different in composition from the dyspeptone formed in gastric digestion. In one experiment, where about two kilograms of pure, moist casein were heated with two litres of water and 100 grams of concentrated sulphuric acid for seven hours

at 100° C., and the residue so obtained treated again in a like manner with the same strength of acid, a comparatively large amount of casein antialbumid was obtained, which unlike the dyspeptone from a gastric digestion, was only slowly soluble in dilute sodium carbonate. Freed from any adhering soluble products by treatment with several litres of a vigorous gastric juice for two days at 40° C., it still dissolved slowly in dilute sodium carbonate. By long contact with a one per cent. solution of the alkaline carbonate it finally dissolved, leaving but a small residue. From this solution, the antialbumid was reprecipitated by hydrochloric acid, and after thorough washing with water, it was again dissolved in sodium carbonate, the fluid made exactly neutral and then dialyzed until all chloride was removed. After concentration of the fluid and precipitation with alcohol, etc., the antialbumid was dried and analyzed. It contained 18 per cent. of ash. The ash-free substance contained 54·4 per cent. of carbon, 6·8 per cent. of hydrogen, and 14·8 per cent. of nitrogen; showing thus a much higher percentage of carbon, and a lower percentage of nitrogen than the dyspeptone formed by pepsin-hydrochloric acid.

Both casein antialbumid and dyspeptone are dissolved with more or less readiness by alkaline solution of trypsin and are converted by long warming at 40° C. into a peptone-like body, presumably antipeptone.

II. *Caseoses;*— from experiments by *Charles Norris, Jr., Ph.B., and C. A. Tuttle, Ph.B.*

In a previous study of the caseoses formed in pepsin digestion,* we were much impressed with the peculiar behavior of protocaseose towards acids. Unlike the proto bodies from other proteids previously studied, *aqueous* solutions of the substance gave heavy precipitates with dilute acids. Protocaseose, as then separated, was readily soluble in 0·4 per cent. hydrochloric acid, but addition of stronger acid invariably produced a decided precipitate, soluble, however, in a still larger excess of acid. This peculiarity rendered the protocaseose an object of some interest to us, and further study of the conditions favoring its formation in gastric digestion has shown us that, apparently, the nature of the body precipitated by saturation with salt, as well as the body precipitated by salt-saturated acetic acid is modified by the strength of the pepsin solution,

* Chittenden and Painter. Studies from Laboratory of Physiological Chemistry, vol. ii, p. 195.

and the length of time the casein is subjected to the action of the ferment. In our earlier work with the proteoses, we deemed it essential in attempting a study of the primary products of proteolytic action to use as weak a ferment solution as possible, and to discontinue its action as soon as solution of the proteid was complete, in order that there might not be too great a loss through formation of peptone. Further study, however, especially by use of the ammonium sulphate reaction, has shown us that the formation of peptone is a far less rapid process than generally supposed. Indeed, in the majority of artificial digestions with pepsin-hydrochloric acid, as ordinarily conducted, the ammonium sulphate reaction will show the entire absence of peptone. True peptone appears to be formed only by the action of a very vigorous pepsin mixture and that long continued.

In all of our previous experiments, the casein was either subjected to the action of a very weak pepsin mixture or else, in the use of a stronger ferment, exposed to its action for a few hours only. We now find that by using a far more vigorous pepsin mixture and by continuing its action for several days instead of hours, there is still not a trace of peptone to be found in the filtrate from the ammonium sulphate precipitate of the caseoses, but that the caseoses themselves, particularly the proto and deuterocaseose, differ somewhat, both in composition and reactions from the products previously separated. The discovery of this fact has led us to a further study of the caseoses formed in pepsin digestion, by which we have been able in many ways to verify our former observations and at the same time extend our knowledge of these interesting primary cleavage products of casein. We have also extended our work by studying the caseoses formed through the action of trypsin, and dilute sulphuric acid.

### *A. Caseoses formed by pepsin-hydrochloric acid.*

In all of these experiments the pepsin mixture was very powerful, and was especially prepared to insure freedom from both albumoses and peptone.* The casein was, likewise, thoroughly pure, having been freshly prepared from skim milk by precipitation with 0·2 per cent. hydrochloric acid, and reprecipitation three or four times after solution in ammoniacal water.

* Studies from the Laboratory of Physiological Chemistry, vol. ii, p. 133.

*Digestion A.*

Nearly 2 kilos. of moist casein were warmed at 40° C. for a little more than two days, with 10 litres of 0·4 per cent. hydrochloric acid containing sufficient of the pepsin mixture to insure vigorous action. After partial neutralization of the acid, the clear fluid was filtered from the semi-gelatinous dyspeptone, made exactly neutral with sodium hydroxide and then evaporated until moderately concentrated. On filtering the concentrated fluid through paper, a small residue remained, somewhat gummy, insoluble in dilute acid, but readily soluble in dilute sodium carbonate, from which it was precipitated by either hydrochloric or acetic acid. The amount was too small for study, but it seemed to resemble in reactions casein dyspeptone.

The neutral fluid containing the caseoses gave no precipitate whatever on addition of 0·4 per cent. hydrochloric acid or even stronger acid, and in this respect differs from the earlier digestions in which the ferment action was continued for a short time only. With dilute acetic acid, however, a slight turbidity was produced, the amount of which was too small to admit of any study of its character.

The caseoses were precipitated collectively in the form of a heavy gummy precipitate, by saturation of the neutral fluid with ammonium sulphate. On boiling the filtrate from this ammonium sulphate precipitate, a small quantity of a second gummy precipitate was obtained. In the filtrate from this second precipitate, no trace of a peptone-like body could be discovered by any of the ordinary tests. Apparently only caseose bodies had been formed.

The first and main ammonium sulphate precipitate, after being washed by trituration with a saturated solution of ammonium sulphate, was dissolved in water and the perfectly neutral fluid saturated in the cold with sodium chloride. By this means a heavy gummy precipitate was formed, which after being washed with a saturated solution of sodium chloride was redissolved in water, and reprecipitated by saturation of the neutral fluid with salt. After three or four reprecipitations, the protocaseose was considered sufficiently pure.

In this digestion, there appeared to be present more heterocaseose and dyscaseose than in our former experiments, as was evidenced by the small insoluble residues remaining each time the precipitated protocaseose was redissolved in water. These residues of hetero- and

dyscaseose were very small, but still sufficiently large to enable us to make out their general characters. Similarly, Dr. Thierfelder* found in the purification of his protocaseose, or "propeptone I" as he terms it, a small, insoluble residue each time he dissolved the sodium chloride precipitate in water. It would appear, therefore, that in a vigorous or long continued digestion there is a much greater probability of the hetero body being formed than when the ferment is allowed to act only a short time on the casein. This, however, is contrary to Neumeister's views regarding the order of formation of the proteoses.

In order to free the precipitated protocaseose from salt and any adhering heterocaseose, it was dissolved in water and dialyzed until all chloride was removed. The neutral solution was then concentrated to a syrup, and the proto body precipitated by alcohol. During the concentration of the fluid, a gummy-like mass separated, similar to the separation of protoelastose. This, however, dissolved more or less completely as the mixture cooled. The precipitated caseose, after being extracted several times with alcohol and with warm ether, was partially dried, ground to a fine powder, re-extracted with ether in a fat extractor and finally dried at 110° C. until of constant weight.

On analysis it gave the following results:

*Protocaseose A.*

I. 0·4947 gram substance gave 0·9794 gram $CO_2$=53·98 per cent. C.

II. 0·3802 gram substance gave 0·2397 gram $H_2O$=7·00 per cent. H.

III. 0·3862 gram substance gave 0·2467 gram $H_2O$=7·09 per cent. H and 0·7670 gram $CO_2$=54·15 per cent. C.

IV. 0·4953 gram substance gave 64·8 c. c. N at 20·8° C. and 760·3 mm pressure=15·72 per cent. N.

V. 0·3764 gram substance gave 50·1 c. c. N at 22·5° C. and 760·6 mm pressure=15·95 per cent. N.

VI. 0·4599 gram substance gave 0·0046 gram ash=1·00 per cent.

VII. 0·4103 gram substance gave 0·0041 gram ash=0·99 per cent.

*Percentage composition of ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 54·53 | 54·69 | .... | .... | .... | 54·61 |
| H | .... | 7·11 | 7·07 | .... | .... | 7·11 |
| N | .... | .... | .... | 15·88 | 16·11 | 15·99 |

* Zur Kenntniss der Caseinpeptone, Zeitschrift für physiologische Chemie, x, p. 577.

This protocaseose is thus seen to have a higher percentage of carbon than the proto bodies previously* studied, although one product was then obtained with 53·93 per cent. C, 7·17 per cent. H, and 16·05 per cent. N.

In reactions, likewise, this protocaseose differs somewhat from the protocaseoses previously obtained, and as the characters of the present body have been verified by the reactions of several other products similarly produced, we are led to believe in their constancy. Moistened with water, the powdered protocaseose becomes immediately gummy and soon dissolves to a perfectly clear fluid, which on addition of considerable water becomes decidedly cloudy or turbid. Treated with a large amount of water at the outset, the protocaseose dissolves more slowly, giving a more or less turbid fluid. In dilute acid and in dilute sodium carbonate it dissolves to a perfectly clear fluid.

Towards heat, aqueous solutions of protocaseose act exactly like protoelastose. Even when warmed very gently, the solution becomes quickly turbid and if concentrated gives more or less of a flocculent precipitate. On cooling, the turbidity disappears, reappearing as the fluid is heated. Like protoelastose also, a solution of the caseose body on being rapidly concentrated deposits more or less of the substance as a gummy mass, which, however, will dissolve in cold water, or if the fluid is not too concentrated will dissolve in the mother liquid as it cools. Dilute acetic acid added to an aqueous solution of protocaseose gives no precipitate whatever, but potassium ferrocyanide added to the acid fluid gives a heavy precipitate.

Dilute hydrochloric acid produces no precipitate.

Dilute nitric acid added to an aqueous solution of the caseose gives a heavy white precipitate, which on gently warming, quickly dissolves while the fluid takes on a faint pink or rose color, which on further warming changes to a bright yellow or reddish yellow color. If the nitric acid solution is not warmed too long, the precipitate reappears as the mixture cools.

Solution of cupric sulphate gives a heavy, greenish white precipitate when added to an aqueous solution of protocaseose.

As already stated, the first precipitates of protocaseose obtained by saturating the neutralized digestive fluid with salt, were not entirely soluble in water; a small residue remained, apparently

* See Chittenden and Painter. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 197.

wholly insoluble. This residue, which by analogy should consist of hetero and dyscaseose was somewhat soluble in salt solution and wholly soluble in dilute sodium carbonate and dilute acid, both acetic and hydrochloric, even dissolving in 0·2 per cent. hydrochloric acid. It was dissolved in sodium carbonate, the fluid neutralized without giving any precipitate, and dialyzed until the sodium chloride was entirely removed. The neutral and clear fluid was then concentrated to a syrup, precipitated by alcohol and the precipitate dried at 110° C. On treatment with cold water, it was now found soluble to a large extent, though a certain amount of gummy matter was still insoluble. The soluble portion showed all of the reactions of the proto body; the solution being rendered turbid by heat, clear again on cooling, and giving in the cold a heavy precipitate with nitric acid, soluble when heated. This behavior of the insoluble heterocaseose towards dilute sodium carbonate would seem to imply a conversion of this substance into protocaseose, or perhaps a reconversion of the coagulated heterocaseose dyscaseose, into heterocaseose proper.

*Acetic acid precipitate.*

On adding a little 30 per cent. acetic acid to the original salt-saturated filtrate from the first sodium chloride precipitate of protocaseose, etc., a heavy, flocculent precipitate settled out, which in amount far exceeded the protocaseose and which on standing, soon became gummy. Excess of acid was avoided, as the precipitate was somewhat soluble in a large amount of the reagent. The gummy mass, after being washed as thoroughly as possible with saturated salt solution, was treated with cold water, in which the greater portion of the substance dissolved, the solution made neutral, dialyzed, concentrated to a syrup, and the substance precipitated with alcohol. It was then thoroughly extracted with ether and finally dried at 110° C.

In reactions, it differed decidedly from protocaseose; in water it was quickly and completely soluble and the solution when heated gave no coagulum whatever, or at the most only the slightest approach to a turbidity.

Dilute nitric acid, in the cold, gave no precipitate; when heated, the acid fluid changed to a reddish yellow color, which quickly turned yellow.

With acetic acid, an aqueous solution of the substance remained perfectly clear. Potassium ferrocyanide, however, when added to the acid fluid gave a heavy precipitate.

Saturation of the aqueous solution with salt gave a slight turbidity.

Cupric sulphate gave a heavy precipitate, soluble in excess.

On analysis, the substance gave the following results, which show a composition quite different from that of protocaseose.

### *Acetic Acid Precipitate A.*

I. 0·4629 gram substance gave 0·2812 gram $H_2O$=6·74 per cent. H and 0·8608 gram $CO_2$=50·71 per cent. C.

II. 0·3277 gram substance gave 0·1977 gram $H_2O$=6·70 per cent. H and 0·6050 gram $CO_2$=50·34 per cent. C.

III. 0·4253 gram substance gave 53·1 c. c. N at 13·0° C. and 761·[illegible] mm pressure=15·01 per cent. N.

IV. 0·4843 gram substance gave 60·8 c. c. N at 13·0° C. and 760·8 mm pressure=15·07 per cent. N.

V. 0·3649 gram substance gave 0·0108 gram ash=2·96 per cent.

VI. 0·3947 gram substance gave 0·0124 gram ash=3·14 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 52·30 | 51·92 | .... | .... | *52·10* |
| H | 6·96 | 6·90 | .... | .... | *6·93* |
| N | .... | .... | 15·48 | 15·54 | *15·51* |

As previously stated, the above original precipitate produced by acetic acid was not entirely soluble in water. A small residue remained, which after being washed with water was dissolved in dilute sodium carbonate, and the solution neutralized with dilute hydrochloric acid, without yielding any neutralization precipitate. The solution was then dialyzed, concentrated to a syrup and the substance precipitated by alcohol. It was now found, to a great extent, soluble in water, the solution showing no turbidity by heat and giving no precipitate with nitric acid. Cupric sulphate gave a heavy precipitate, and dilute acetic acid added to the aqueous solution produced quite a heavy precipitate, not readily soluble in excess of the acid. The amount of substance was too small to admit of analysis, and the reactions are hardly sufficient to identify it. It is evidently not heterocaseose, for it is only the acetic acid compound that is insoluble in water, not the caseose substance itself. In many respects it appears like casein dyspeptone, and as this substance is precipitated by saturation of its aqueous solution with ammonium sulphate and not by sodium chloride, the presence of a trace of this body might not be impossible.

*Ammonium sulphate precipitate.*

On adding ammonium sulphate in substance to the above salt-saturated acetic acid fluid, a slight gummy precipitate was obtained, readily and completely soluble in water, and which, after removal of the salts by dialysis and concentration of the fluid, was precipitated by alcohol. In reactions it did not differ materially from the body obtained by precipitation with acetic acid, except that with cupric sulphate only a slight precipitate was produced. With acetic acid and potassium ferrocyanide, on the other hand, a distinct precipitate was obtained, while with nitric acid in the cold no turbidity whatever was produced.

In composition, however, it differed decidedly from the preceding preparations, although as it contained considerable ash, nearly 10 per cent., the result perhaps can be considered only as an approximation to the truth.

Following is the percentage composition of the ash-free substance:

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 48.33 | 48·18 | .... | .... | *48·23* |
| H | 6·88 | 7·01 | .... | .... | *6·94* |
| N | .... | .... | 15·63 | 15·75 . | *15·69* |

This body, which by analogy should be nearly pure deutero-caseose, evidently approaches much nearer to our conception of a true casein peptone than any of the preceding preparations. Another body, however, has been obtained with a still lower content of carbon and with reactions still more closely approximating to true peptone.

In the first precipitation of the caseoses from this digestion by saturation of the original fluid with ammonium sulphate, it will be remembered that a small amount of a second gummy precipitate was obtained on heating the cold saturated ammonium sulphate filtrate. This gummy precipitate of a caseose body, after purification by dialysis and precipitation with alcohol, was found to consist of a substance extremely soluble in water, the solution giving no precipitate with acetic acid and potassium ferrocyanide, neither with nitric acid nor with cupric sulphate. After being dried at 110° C. it gave on analysis the following results:

I. 0·7280 gram substance gave 0·4239 gram $H_2O$=6·46 per cent. H and 1·2160 grams $CO_2$=45·54 per cent. C.

II. 0·5020 gram substance gave 0·2905 gram $H_2O$=6·40 per cent. H and 0·8442 gram $CO_2$=45·85 per cent. C.

III. 0·3291 gram substance gave 42·1 c. c. N at 13·9° C. and 756 mm pressure=15·33 per cent. N.

IV. 0·6835 gram substance gave 87·0 c. c. N at 14·5° C. and 756 mm pressure=15·30 per cent. N.

V. 0·5370 gram substance gave 0·0230 gram ash=4·28 per cent.

VI. 0·3525 gram substance gave 0·0154 gram ash=4·36 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 47·57 | 47·87 | .... | .... | *47·72* |
| H | 6·75 | 6·70 | .... | .... | *6·73* |
| N | .... | .... | 15·99 | 15·96 | *15·97* |

This substance, since it is precipitable by ammonium sulphate, cannot be considered a true peptone, yet in composition it closely approaches both the ampho- and antipeptone from fibrin,* which it also resembles somewhat in reactions, except in its behavior towards ammonium sulphate.

It is thus evident from the foregoing, that in this active and comparatively long continued digestion there is a much smaller amount of protocaseose present than was found in our former experiments. Indeed, deuterocaseose appears to predominate, while at the same time the protocaseose is modified both in composition and reactions, due in part without doubt, to adhering heterocaseose. Further, we are inclined to consider the presence of at least two forms of deuterocaseose. We have generally considered that a proto body is never completely precipitated by saturation of its aqueous solution with salt, and that consequently the precipitate produced by acid in the salt-saturated fluid must be a mixture of proto and deuteroproteose, and this we have usually found to be the case. In the present digestion, however, the acetic acid precipitate contained only a very small amount of protocaseose, for as previously stated this precipitate when purified gave only a slight turbidity on saturation of its aqueous solution with salt and no precipitate whatever with nitric acid; both of which reactions would indicate freedom from any large amount of protocaseose. This view is further substantiated by the great difference in the percentage of carbon of the two bodies. To be sure we have, with Neumeister, looked on the cupric sulphate reaction as a means of distinguishing between proto and deuteroalbumose, but it does not necessarily follow that the same reaction will hold good for all proteoses. The acetic acid precipitate does indeed give a strong

* Kühne and Chittenden, Studies, vol. ii, p. 40.

reaction with cupric sulphate, but the striking differences in composition and reactions between the purified sodium chloride precipitate and the acetic acid precipitate (when freed from acid) point to a totally different nature, and we are inclined to consider the latter as a deutero body, probably contaminated with a little protocaseose, and for convenience we propose to call it *a* deuterocaseose.

It is unquestionably very difficult, if not almost impossible, to isolate the individual caseoses in a state of perfect purity. Whenever one is precipitated, it usually brings down with it more or less of any other caseose present and such admixtures are very hard to remove. It is, we think, owing to this fact that we have not been able to obtain *a* deuterocaseose sufficiently free from protocaseose as not to give any precipitate on saturation with sodium chloride.

That form of caseose in this digestion which was not precipitated by salt, or by salt and acetic acid, but which appeared on addition of ammonium sulphate in the cold, is probably a mixture of *a* deutero and and what we term *β* deuterocaseose, with possibly an intermediate body.

The name *β* deuterocaseose, we apply to that caseose not readily precipitable by saturation with ammonium sulphate in the cold, and which is generally found in a greater or less quantity in the filtrate from the precipitate produced by saturation with ammonium sulphate. It is precipitated fairly pure, as a sticky gum, by simply boiling the saturated ammonium sulphate filtrate and is especially characterized by its low content of carbon, and by its non-precipitation with acetic acid and potassium ferrocyanide, with nitric acid, and with cupric sulphate. It stands, unquestionably, nearer to peptone than *a* deuterocaseose and is doubtless formed from the latter by the continued action of the ferment.

### *Digestion B.*

In this digestion, 2 kilos. of moist casein were warmed at 40° C. for eight days, with about 5 litres of 0·4 per cent. hydrochloric acid containing an active pepsin solution, after which the mixture was partially neutralized with sodium carbonate and filtered from the dyspeptone. The clear fluid was then made exactly neutral (no neutralization precipitate) and concentrated to a thin syrup. When cold, proto and heterocaseose were directly precipitated by saturation of the solution with salt. Protocaseose was purified by repeated precipitation with salt, etc., as described under *A*. In this process, the same insoluble residues of hetero and dyscaseose were met with

as in the previous digestion. The purified protocaseose showed the same peculiar reactions as protocaseose *A.*, viz: with nitric acid a heavy white precipitate, soluble on warming; with heat alone, a distinct turbidity or coagulum, disappearing as the solution cooled; and giving with a large amount of water a more or less turbid fluid, from which on standing a little gummy matter separated.

Dried at 110° C. and analyzed, the following results were obtained.

*Protocaseose B.*

I. 0·3470 gram substance gave 0·2192 gram $H_2O$=7·02 per cent. H.

II. 0·2935 gram substance gave 0·1854 gram $H_2O$=7·01 per cent. H and 0·5779 gram $CO_2$=53·69 per cent. C.

III. 0·2952 gram substance gave 0·5853 gram $CO_2$=54·07 per cent. C.

IV. 0·2989 gram substance gave 38·6 c. c. N at 13·8° C. and 760·1 mm pressure=15·52 per cent. N.

V. 0·5564 gram substance gave 72·8 c. c. N at 13·1° C. and 760·1 mm pressure=15·69 per cent. N.

VI. 0·3594 gram substance gave 0·0047 gram ash=1·30 per cent.

VII. 0·3523 gram substance gave 0·0045 gram ash=1·27 per cent.

*Percentage composition of ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | .... | 54·77 | 54·89 | .... | .... | 54·58 |
| H | 7·10 | 7·09 | .... | .... | .... | 7·10 |
| N | .... | .... | .... | 15·71 | 15·90 | 15·80 |

In composition, therefore, as in reactions, this body is apparently identical with protocaseose *A*.

In the original salt-saturated filtrate from protocaseose, acetic acid produced a heavy, gummy precipitate, which was dissolved in water and purified in the same manner as the corresponding body in digestion *A*. Like the latter, it was readily and completely soluble in water, the solution giving no turbidity whatever by heat, nor on the addition of either nitric or acetic acid. With cupric sulphate, a heavy precipitate was formed, as also with acetic acid and potassium ferrocyanide.

Dried at 110° C. it gave the following results on analysis:

*Acetic acid precipitate B.*

I. 0·3429 gram substance gave 0·2057 gram $H_2O$=6·66 per cent. H and 0·6315 gram $CO_2$=50·22 per cent. C.

II. 0·4345 gram substance gave 0·2734 gram $H_2O$=6·72 per cent. H and 0·8358 gram $CO_2$=50·48 per cent. C.

III. 0·3316 gram substance gave 43·5 c. c. N at 13·7° C. and 756·3 mm pressure=15·70 per cent. N.

IV. 0·3258 gram substance gave 43·1 c. c. N at 13·2° C. and 759·1 mm pressure=15·86 per cent. N.

V. 0·3306 gram substance gave 0·0126 gram ash=3·81 per cent.

VI. 0·2262 gram substance gave 0·0085 gram ash=3·76 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 52·17 | 52·43 | ---- | ---- | *52·30* |
| H | 6·92 | 6·99 | ---- | ---- | *6·95* |
| N | ---- | ---- | 16·32 | 16·49 | *16·40* |

This body appears to differ from *a* deuterocaseose obtained in digestion *A* by nearly 1 per cent. of nitrogen, but in all other respects is practically identical with it.

As in digestion A, the precipitate first obtained by the addition of acetic acid was not entirely soluble in water, a residue remained soluble in dilute sodium carbonate, and which comported itself exactly like the insoluble residue obtained in the preceding digestion, apparently being a trace of casein dyspeptone.

The original filtrate from the above acetic acid precipitate, on saturation with ammonium sulphate, gave an additional precipitate, hardly sufficient for analysis, but which when purified proved to be identical with the corresponding body from *A* and like it giving with cupric sulphate only a very slight precipitate.

It is thus seen that in a vigorous pepsin digestion of casein there are formed, in addition to dyspeptone and heterocaseose, at least three distinct caseoses all soluble and differing from each other both in composition and reactions. As compared with casein, protocaseose is somewhat peculiar in containing a higher percentage of carbon than the mother substance. All of the other products show a very much smaller content of carbon. The relative composition of the products is shown in the following table:

| | *Casein.* | *Proto-caseose A.* | *Proto-caseose B.* | *a Deutero-caseose A.* | *a Deutero-caseose B.* | *β Deutero-caseose A.* |
|---|---|---|---|---|---|---|
| C | 53·30 | 54·61 | 54·58 | 52·10 | 52·30 | 47·72 |
| H | 7·07 | 7·11 | 7·10 | 6·98 | 6·95 | 6·78 |
| N | 15·91 | 15·99 | 15·80 | 15·51 | 16·40 | 15·97 |

Protocaseose agrees closely in its content of carbon with the "propeptone I" of Thierfelder.* This investigator has separated from a pepsin digestion of casein, by a process similar to our method of separating the proto body, a substance to which he gives the above name and which contained 54·63 per cent. of carbon and 7·45 per cent. of hydrogen. In reactions it was similar to protocaseose, except that aqueous solutions of the substance remained perfectly clear on warming. Nitrogen was not determined. Thierfelder also separated from the filtrate from his "propeptone I," a second body, by addition of hydrochloric acid, to which he gives the name "propeptone II." This substance, which corresponds to our *a* deuterocaseose, he found to contain 49·8 per cent. carbon, 7·18 per cent. hydrogen and 14·23 per cent. nitrogen. Judging from the method of separation, however, the body analyzed must have been an acid compound of the caseose and not the caseose body itself. In reactions, so far as they are given, the substance was not different from *a* deuterocaseose.

### *b. Caseoses from Weyl's casein peptone.*

This commercial product, sent to us from Germany, we have examined according to the foregoing methods and have found it, as might be expected, composed almost entirely of caseoses. It was completely soluble in water and gave with acetic acid a slight turbidity, somewhat increased by addition of potassium ferrocyanide. By saturation of its aqueous solution with sodium chloride, only a comparatively small precipitate was obtained, greatly increased, however, by addition of acetic acid.

200 grams of the powder were dissolved in water and the caseoses precipitated collectively by saturation of the fluid with ammonium sulphate, in the form of a heavy gummy precipitate. On heating the filtrate from this precipitate of caseoses until a crust of ammonium sulphate formed on the surface of the hot fluid, a second gummy precipitate gradually separated, which after purification by dialysis, etc., was finally precipitated by alcohol, and a portion dried at 110° C. for analysis.

This substance, representing a form of caseose not readily precipitable by ammonium sulphate and thus indicating its close approach to true peptone, is apparently identical with the $\beta$ deuterocaseose similarly obtained in our own digestion, but present here in much

* Zeitschrift für physiologische Chemie, Band x, p. 585.

larger quantity. In water it was readily and completely soluble, the solution giving no turbidity whatever by heat, nor with dilute nitric acid. With cupric sulphate only a very slight turbidity was produced, and with acetic acid and potassium ferrocyanide a correspondingly slight turbidity. Its aqueous solution on being saturated with salt and then made slightly acid with acetic acid showed a small flocculent precipitate, doubtless representing the substance which gave the slight turbidity with cupric sulphate and potassium ferrocyanide, viz: *a* deuterocaseose. On analysis the following results were obtained:

*β deuterocaseose from Weyl's casein peptone.*

I. 0·3641 gram substance gave 0·2121 gram $H_2O$=1·47 per cent. H and 0·6087 gram $CO_2$=45·58 per cent. C.

II. 0·4600 gram substance gave 0·2708 gram $H_2O$=6·54 per cent. H and 0·7652 gram $CO_2$=45·36 per cent. C.

III. 0·3298 gram substance gave 42·2 c. c. N at 13·9° C. and 755·4 mm pressure =15·31 per cent. N.

IV. 0·3418 gram substance gave 43·5 c. c. N at 14·4° C. and 756·1 mm pressure =15·31 per cent. N.

V. 0·3514 gram substance gave 0·0153 gram ash =4·35 per cent.

VI. 0·3599 gram substance gave 0·0155 gram ash =4·30 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 47·61 | 47·40 | ---- | ---- | *47·50* |
| H | 6·76 | 6·83 | ---- | ---- | *6·79* |
| N | ---- | ---- | 16·00 | 15·84 | *15·92* |

In composition, therefore, as well as in reactions, this body resembles the *β* deutero described under *A*, and like it is especially characterized by its exceedingly low percentage of carbon.

From the first ammonium sulphate precipitate of caseoses, protocaseose was separated by saturation of the aqueous solution of the above precipitate with salt. As in similar precipitates from the preceding digestions, there was considerable heterocaseose present, showing itself as an insoluble residue when the sodium chloride precipitate was dissolved in water for reprecipitation. Purified after the methods previously described, the protocaseose showed the usual reactions characteristic of this body, its aqueous solution growing turbid when heated, giving a precipitate with nitric acid, etc.

Dried at 110° C. it gave on analysis the following results:

*Protocaseose, from Weyl's casein peptone.*

I. 0·4291 gram substance gave 0·2597 gram $H_2O$=6·72 per cent. H and 0·8140 gram $CO_2$=51·70 per cent. C.

II. 0·3647 gram substance gave 0·2155 gram $H_2O$=6·56 per cent. H and 0·6935 gram $CO_2$=51·85 per cent. C.

III. 0·4115 gram substance gave 52·5 c. c. N at 13·6° C. and 760·8 mm pressure=15·11 per cent. N.

IV. 0·7730 gram substance gave 96·9 c. c. N at 13·8° C. and 761·2 mm pressure=15·10 per cent N.

V. 0·2901 gram substance gave 0·0121 gram ash=4·16 per cent.

VI. 0·2959 gram substance gave 0·0120 gram ash=4·05 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 53·93 | 54·08 | .... | .... | *54·01* |
| H | 7·01 | 6·84 | .... | .... | *6·92* |
| N | .... | .... | 15·75 | 15·70 | *15·72* |

Addition of salt-saturated acetic acid to the filtrate from protocaseose, gave a moderately heavy, gummy precipitate which, after purification and removal of the acid, was completely soluble in water, the solution showing no turbidity by heat, but giving a slight turbidity with dilute nitric acid. It gave all of the reactions mentioned as characteristic of this precipitate. It was not analyzed.

The original filtrate from the acetic acid precipitate gave on saturation with ammonium sulphate, a small gummy precipitate which after purification was found wholly soluble in water, the solution giving no turbidity by heat and no precipitate with nitric or acetic acid. With acetic acid and potassium ferrocyanide, however, a distinct turbidity was produced and with cupric sulphate a heavy precipitate. The substance was not analyzed.

It is thus evident that Weyl's so-called casein peptone, however it may be prepared, contains essentially the same kind of caseose found in our own digestions with pepsin-hydrochloric acid. In addition, a small amount of a substance was found not precipitable by saturation with ammonium sulphate and which gave no precipitate with nitric or acetic acid, nor with acetic acid and potassium ferrocyanide, and with cupric sulphate only a slight turbidity. This body, which doubtless was amphopeptone mixed with a little caseose, was obtained in too small quantity for analysis.

*c. Caseoses formed by dilute sulphuric acid at* 100° *C.*

Two kilos. of pure moist casein were heated in a flask with 2 litres of water and 100 grams of pure sulphuric acid at 100° C. for seven hours. The residue of casein and antialbumid was again warmed with a like amount of fresh acid and water for six hours. The acid fluids were united, neutralized with sodium carbonate without giving any noticeable precipitate, and then evaporated until moderately concentrated. On cooling, considerable tyrosin and leucin crystallized from the fluid. With cupric sulphate, the solution gave a heavy precipitate which dissolved in sodium hydroxide with a reddish color. Sodium chloride and ammonium sulphate both produced heavy precipitates when added to saturation.

The caseoses were separated from the fluid by saturation in the cold with ammonium sulphate, and from this precipitate, protocaseose was separated by solution in water and precipitation with sodium chloride. The sodium chloride precipitate was dissolved in water, the substance reprecipitated by saturation with salt, and again dissolved in water to which a trace of sodium carbonate was added to make the mixture quite neutral. On dialysis, a small amount of gummy heterocaseose separated, mixed with a little tyrosin. The clear fluid, now free from salts, was concentrated on a water-bath. As the evaporation advanced, a brown gummy mass settled out, which was, however, readily soluble in cold water, for as the concentrated fluid cooled at night the gummy matter entirely disappeared, reappearing as the fluid was again heated. The final concentrated fluid was treated with alcohol, the gummy precipitate boiled with alcohol repeatedly to free it from any adhering tyrosin, and finally extracted with ether and dried at 110° C.

Analyzed, it gave the following results:

*Protocaseose, formed by sulphuric acid.*

I. 0·4405 gram substance gave 0.2718 gram $H_2O$=6·85 per cent. H and 0·8756 gram $CO_2$=54·20 per cent. C.

II. 0·3783 gram substance gave 0·2341 gram $H_2O$=6·87 per cent. H and 0.7604 gram $CO_2$=54·81 per cent. C.

III. 0·3786 gram substance gave 48·3 c. c. N at 17·5° C. and 753·4 mm pressure=14·93 per cent. N.

IV. 0·3780 gram substance gave 47·8 c. c. N at 17·0° C. and 754·0 mm pressure=14·84 per cent. N.

V. 0·3701 gram substance gave 0·0113 gram ash=3·05 per cent.

VI. 0·5112 gram substance gave 0·0154 gram ash=3·02 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 55·90 | 56·50 | .... | .... | *56·20* |
| H | 7·07 | 7·09 | .... | .... | *7·08* |
| N | .... | .... | 15·40 | 15·81 | *15·56* |

After being dried at 110° C. the substance dissolved with difficulty in water, leaving a large residue, but was readily and completely soluble in 0·2 per cent. hydrochloric acid and in 0·2 per cent. sodium carbonate. From the alkaline solution it was reprecipitated by hydrochloric acid, and not readily dissolved by an excess of the acid. Aqueous solution of the substance was rendered turbid by heat, the turbidity disappearing as the solution cooled. With dilute nitric acid, a white precipitate was formed, readily soluble on warming and reappearing as the solution cooled. Acetic acid and potassium ferrocyanide gave a heavy precipitate.

Thus in many respects this body resembles protocaseose formed by pepsin-hydrochloric acid, but is apparently characterized by a somewhat higher percentage of carbon.

The filtrate from the original sodium chloride precipitate, treated with salt-saturated 30 per cent. acetic acid, gave a flocculent, changing to gummy precipitate which was readily and completely soluble in water. The solution made exactly neutral was dialyzed without showing any evidence of a hetero-like body, finally concentrated and precipitated by alcohol. After being dried at 110° C., the substance was only partially soluble in water. The aqueous solution was rendered turbid by heat, clear again on cooling, and gave with nitric acid a heavy white precipitate as also with acetic acid and potassium ferrocyanide.

Analyzed, it gave the following results:

*Acetic acid precipitate of caseose (a deuterocaseose?) formed by dilute sulphuric acid.*

I. 0·4056 gram substance gave 0·2383 gram $H_2O$=6·52 per cent. H and 0·7733 gram $CO_2$=51·99 per cent. C.

II. 0·4697 gram substance gave 0·2740 gram $H_2O$=6·48 per cent. H and 0·8910 gram $CO_2$=51·73 per cent. C.

III. 0·5379 gram substance gave 66·5 c. c. N at 14·9° C. and 752·1 mm pressure=14·58 per cent. N.

IV. 0·4150 gram substance gave 0·0210 gram ash=5·06 per cent.

V. 0·5136 gram substance gave 0·0247 gram ash=4·80 per cent.

*Percentage composition of ash-free substance.*

| | | | | Average. |
|---|---|---|---|---|
| C | 54·68 | 54·42 | .... | *54·55* |
| H | 6·86 | 6·81 | .... | *6·84* |
| N | .... | .... | 15·33 | *15·33* |

This caseose, instead of being a pure deutero body, appears to be a mixture of proto and *a* deutero as indicated by its behavior towards heat and nitric acid; indeed, the behavior of this acetic acid precipitate resembles the acetic acid precipitate of caseose obtained in our previous work, where the two bodies were plainly precipitated together. In composition, while it agrees closely with protocaseose formed by pepsin-hydrochloric acid, it has a higher content of carbon than the corresponding *a* deutero, but bears about the same relation to the sulphuric acid protocaseose as the *a* deutero formed by pepsin to its corresponding protocaseose.

On boiling the original ammonium sulphate-saturated filtrate, a second gummy precipitate gradually separated from the hot fluid. This caseose, after purification by the usual methods and drying at 110° C., was entirely soluble in hot and cold water, and was especially characterized by yielding with acetic acid a heavy white precipitate, soluble in excess of the acid. With nitric acid it also gave a white precipitate, soluble in excess of acid. Cupric sulphate likewise gave a heavy precipitate. The cold water solution was not rendered turbid by heat.

Analyzed it gave the following results:

*β deuterocaseose, formed by dilute sulphuric acid.*

I. 0·3952 gram substance gave 0·2308 gram $H_2O$=6·49 per cent. H and 0·7282 gram $CO_2$=50·25 per cent. C.

II. 0·3518 gram substance gave 0·2064 gram $H_2O$=6·51 per cent. H and 0·6440 gram $CO_2$=49·92 per cent. C.

III. 0·4727 gram substance gave 59·2 c. c. N at 15·0° C. and 754·9 mm pressure = 14·81 per cent. N.

IV. 0·4279 gram substance gave 0·0233 gram ash = 5·44 per cent.

V. 0·5184 gram substance gave 0·0219 gram ash = 5·38 per cent.

*Percentage composition of ash-free substance.*

| | | | | Average. |
|---|---|---|---|---|
| C | 53·10 | 52·77 | .... | *52·93* |
| H | 6·86 | 6·89 | .... | *6·87* |
| N | .... | .... | 15.66 | *15·66* |

Thus, this body, which corresponds to the $\beta$ deuterocaseose formed by pepsin-acid, has a relatively higher content of carbon and also varies in certain of its reactions, which resemble rather those of $\alpha$ deutero, and even the protocaseose of a pepsin digestion, than those of a genuine $\beta$ deutero.

Altogether, the three caseoses separated from the sulphuric acid solution of casein, while showing a certain general relationship to the caseoses formed by pepsin-hydrochloric acid, are sufficiently different in their individual reactions to suggest at least some difference in their nature.

### *d. Caseoses formed by the action of trypsin.*

In subjecting casein to the action of trypsin, care was taken that the ferment solution should be as free as possible from all products of the self-digestion of the pancreatic tissue. Dried pancreas from the ox, prepared according to Kühne's method, was warmed with 0·1 per cent. salicylic acid at 40° C. for 24 hours, after which the acid extract was neutralized and made alkaline with sodium carbonate to the extent 0·3 per cent. The alkaline solution, well thymolized, was warmed at 40° C. for several days in order to convert the albuminous matters present into easily diffusible products, after which it was dialyzed in running water for some time, and the solution ultimately evaporated to dryness at 40° C. This residue, being extracted with a small volume of water, gives a fairly pure solution of trypsin, free from objectionable impurities.

In the formation of the caseoses, 2200 grams of pure, moist casein were soaked in 3 litres of 1 per cent. sodium carbonate for several days, and the mixture well thymolized to prevent putrefaction. The trypsin solution was then added, together with some water, and the whole warmed at 40° C. At first, the mixture was quite limpid, the casein being dissolved in the alkaline fluid, but after two or three days, gelatinous lumps began to appear on the bottom of the dish and finally a soft coagulum appeared on the surface and all through the mixture, resembling the separation of antialbumid. This coagulum gradually disappeared and at the end of five days the digestion was stopped, and the alkaline fluid filtered from the undigested residue. The latter was thoroughly washed with thymolized water and the washings added to the filtrate. This residue of undigested matter was found to be insoluble in 0·5 per cent. sodium carbonate and also in 0·2 per cent. hydrochloric acid, but on being warmed with pepsin-hydrochloric acid it was in time almost completely dissolved.

For separation of the caseoses the digestive fluid was neutralized with dilute hydrochloric acid, giving only a slight neutralization precipitate, and the neutral fluid concentrated. When moderately concentrated, the solution was placed in a cool place for several days to allow as much of the leucin and tyrosin to crystallize as possible. The caseoses were then separated from the filtrate, in the form of a gummy precipitate, by saturation of the fluid with ammonium sulphate. On boiling the filtrate from the first precipitate, and adding still more ammonium sulphate, a second gummy precipitate gradually settled out of the hot saturated fluid. This precipitate was separated from the fluid, the latter being saved for the detection of any peptone formed, washed with hot saturated ammonium sulphate solution, then dissolved in water and dialyzed until all traces of sulphate were removed. In the dialysis, no signs of any gummy heterocaseose or other like body was noticed. The caseose was precipitated from the suitably concentrated fluid with alcohol, boiled repeatedly with alcohol to free it from any adhering tyrosin, and finally dried at 110° C.

The substance was extremely soluble in water, the fluid remaining perfectly clear when heated. Acetic acid produced a heavy precipitate, soluble in excess, and in the acid fluid potassium ferrocyanide gave a heavy precipitate. Dilute nitric acid, added to the aqueous solution produced a white precipitate readily soluble in excess of acid, and when heated showed the xanthoprotein reaction.

Cupric sulphate also gave a heavy white precipitate. On analysis, the following results were obtained:

### *β deuterocaseose, formed by trypsin.*

I. 0·3355 gram substance gave 0·1847 gram $H_2O$=6·11 per cent. H and 0·5990 gram $CO_2$=48·68 per cent. C.

II. 0·3459 gram substance gave 0·1871 gram $H_2O$=6·01 per cent. H and 0·6120 gram $CO_2$=48·24 per cent. C.

III. 0·5399 gram substance gave 63·0 c. c. N at 13·8° C. and 762·6 mm pressure = 13·63 per cent. N.

IV. 0·4166 gram substance gave by fusion with KOH+$KNO_3$ 0·0300 gram $BaSO_4$=0·98 per cent. S; after deducting sulphur of ash = 0·95 per cent.

V. 0·5496 gram substance gave by fusion with KOH+$KNO_3$ 0·0380 gram $BaSO_4$=0·95 per cent. S; after deducting sulphur of ash = 0·91 per cent.

VI. 0·4804 gram substance gave 0·0365 gram = 9·59 per cent.

VII. 0·4272 gram substance gave 0·0405 gram ash = 9·43 per cent.

VIII. Ash from 0·8076 gram substance gave 0·0181 gram $BaSO_4$ = 0·03 per cent. S.

*Percentage composition of ash-free substance.*

| | | | | | | Average. |
|---|---|---|---|---|---|---|
| C | 53·81 | 53·31 | ---- | ---- | ---- | *53·56* |
| H | 6·76 | 6.64 | ---- | ---- | ---- | *6·70* |
| N | ---- | ---- | 15·07 | ---- | ---- | *15·07* |
| S | ---- | ---- | ---- | 0·95 | 0·91 | *0·93* |
| O | ---- | ---- | ---- | ---- | ---- | *23·74* |
| | | | | | | *100·00* |

This sample of $\beta$ deutero shows very close agreement in composition and reactions with the like body formed by dilute sulphuric acid; both being characterized by the reaction with acetic acid and showing, by their reactions and composition, a closer relationship to the proto-like bodies than their non-precipitation by ammonium sulphate would appear to warrant. In composition, however, this body shows a much smaller percentage of carbon than the caseose precipitated by acetic acid.

For separation of the other caseoses formed in this digestion, the first ammonium sulphate precipitate was dissolved in water and protocaseose precipitated by saturation of the solution with sodium chloride. The precipitate, which was not very heavy, was purified by reprecipitation and dialysis. On dissolving the first salt precipitate in water quite a little residue was noticed, soluble in dilute sodium carbonate, but readily precipitated by the least trace of acid. The final neutral solution was concentrated, giving when heated a heavy coagulum which finally came together as a gummy mass. The clear fluid, separated from the gum, gave still another coagulum as the heating was continued. On cooling, the gummy matter readily dissolved. As the evaporation continued and the fluid became concentrated the gummy matter dissolved even in the hot fluid, and the caseose was finally precipitated while hot with alcohol, and the precipitate boiled repeatedly with alcohol for the complete removal of leucin and tyrosin.

In reactions, this body was apparently identical with protocaseose formed by pepsin-hydrochloric acid, except that with acetic acid and also with hydrochloric acid it yielded a heavy white precipitate, soluble in excess of acid. In water it dissolved almost completely, the solution, however, becoming turbid when heated and if sufficiently

concentrated giving a gummy deposit as the heating continued. Further, the reaction with heat was the only respect in which this body differed from the preceding ammonium sulphate precipitate. Owing to insufficient quantity it was not analyzed.

In the filtrate from the first sodium chloride precipitate, a second caseose was precipitated by addition of a little 30 per cent. acetic acid. At first it separated as a flocculent precipitate, but on standing changed to a gummy mass, which dissolved more or less readily in water, and completely so when a little sodium carbonate was added to neutral reaction. After reprecipitation, the aqueous solution of the substance was neutralized, dialyzed, the solution concentrated without separation of any gummy matter, and the substance finally precipitated with alcohol. The precipitate was boiled repeatedly with alcohol and finally dried at 110° C. The dried substance was readily soluble in water and also in dilute acetic acid, potassium ferrocyanide producing in the latter solution a slight turbidity only. Added to an aqueous solution of the substance, acetic acid produced a heavy white precipitate readily soluble in excess of the acid. Nitric acid, likewise, produced a heavy white precipitate not so readily soluble in excess. Cupric sulphate also gave a heavy white precipitate. The aqueous solution of the caseose gave no turbidity whatever, when heated.

Analyzed it yielded the following results :

*Acetic acid precipitate of caseose (a deuterocaseose?) formed by trypsin.*

I. 0·5594 gram substance gave 0·3300 gram $H_2O$=6·55 per cent. H and 1·0924 grams $CO_2$=53·25 per cent. C.

II. 0·3670 gram substance gave 0·2157 gram $H_2O$=6·53 per cent. H and 0·7158 gram $CO_2$=53·19 per cent. C.

III. 0·3811 gram substance gave 44·4 c. c. N at 12·8° C. and 765·1 mm pressure =14·07 per cent. N.

IV. 0·2848 gram substance gave 33·0 c. c. N at 13·4° C. and 765·5 mm pressure =13·98 per cent. N.

V. 0·4351 gram substance gave 0·0230 gram ash =5·28 per cent.

VI. 0·4033 gram substance gave 0·0210 gram ash =5·26 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 56·20 | 56·14 | ... | .... | *56·17* |
| H | 6·91 | 6·89 | .... | .... | *6·90* |
| N | .... | .... | 14·85 | 14·75 | *14·80* |

It is thus obvious from the foregoing that by the action of trypsin, caseoses are formed of the same general nature as those formed by the action of pepsin-hydrochloric acid, and by hot dilute sulphuric acid, but with higher contents of carbon.

In other digestions with trypsin, made especially for the preparation of casein peptone, these caseoses were again separated and the foregoing reactions verified. The quantities, however, were too small to admit of their analysis.

III. *Casein peptone;*—from experiments by *Charles Norris, Jr., Ph.B.*

Supersaturation of a digestive fluid with ammonium sulphate, under proper conditions, suffices to entirely remove the preliminary products of proteolytic action. Proper conditions, however, are not obtained by simply adding the ammonium salt to a cold fluid, for as has been already pointed out an additional precipitate of proteose can nearly always be obtained, by heating the cold saturated solution until a thick crust of the ammonium salt forms on the hot fluid. In the present state of knowledge, we assume as peptone any amorphous product of proteolytic action precipitable by alcohol, and not precipitable by heating with ammonium sulphate added to saturation. Unquestionably, the albumose precipitated only by long boiling of a saturated ammonium sulphate solution, is much nearer to true peptone than those bodies more easily precipitated, but at present we are not inclined to accept as true peptone any body precipitable by ammonium sulphate under any conditions whatever. In the present series of experiments we have aimed to prepare a casein peptone, by the action of trypsin, entirely free from albumose in order to study its composition and reactions.

In the digestion of casein with trypsin, described in the preceding section, the filtrate from the ammonium sulphate saturation was heated for some time and the slight gummy film of caseose separated, after which as much of the ammonium sulphate as possible was removed by alternate crystallization, treatment with alcohol, etc. The last traces of the ammonium salt were removed by long continued dialysis in running water, and when finally the fluid gave no reaction with barium chloride it was evaporated to a syrup and precipitated with alcohol. The precipitated peptone was freed from any adhering tyrosin and leucin by repeated treatment with boiling alcohol, and finally dried at 110° C. until of constant weight. This proved a long operation. The peptone was so exceedingly hygroscopic and held on so tenaciously to the water, that it was only after

long-continued drying that a constant weight was reached and then the odor was strongly suggestive of partial dissociation. In a previous article* on peptone, attention was called to the peculiar odor of valerianic acid almost invariably noticed when the fibrin peptone was dried at 110° C., but in that case it was found impossible to bring the product to a constant weight. With casein peptone, the same odor was noticeable on drying the product for analysis, but after a few days heating at 110° C., the weight of the product remained fairly constant.

On analysis, the casein peptone yielded the following results:

*Casein antipeptone A.*

I. 0·5792 gram substance gave 0·3185 gram $H_2O$=6·11 per cent. H and 0·9951 gram $CO_2$=46·85 per cent. C.

II. 0·4692 gram substance gave 0·2562 gram $H_2O$=6·07 per cent. H and 0·8060 gram $CO_2$=46·84 per cent C.

III. 0·4357 gram substance gave 55·5 c. c. N at 14·0° C. and 762·0 mm pressure=15·27 per cent. N.

IV. 0·6534 gram substance gave 83·0 c. c. N at 14·0° C. and 761·0 mm pressure=15·18 per cent. N.

V. 0·9703 gram substance gave 0·0656 gram ash=6·76 per cent.

VI. 0·4939 gram substance gave 0·0326 gram ash=6·60 per cent.

VII. 0·7032 gram substance gave by fusion with $KOH+KNO_3$ 0·0386 gram $BaSO_4$=0·75 per cent. S.

VIII. 0·6526 gram substance gave by fusion with $KOH+KNO_3$ 0·0337 gram $BaSO_4$=0·71 per cent. S.†

*Percentage composition of ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 49·94 | 49·93 | .... | .... | .... | .... | *49·94* |
| H | 6·51 | 6·50 | .... | .... | .... | .... | *6·51* |
| N | .... | .... | 16·35 | 16·26 | .... | .... | *16·30* |
| S | .... | .... | .... | .... | 0·70 | 0·66 | *0·68* |
| O | .... | .... | .... | .... | .... | .... | *26·57* |
| | | | | | | | *100·00* |

Somewhat to our surprise, on testing the purified and dried peptone, we found that its aqueous solution gave a heavy white precipitate with nitric acid, soluble in excess of acid; likewise, a heavy precipitate with acetic acid, also soluble in excess; with cupric sul-

* Kühne and Chittenden, Peptone. Studies, vol. ii.

† The ash contained only a slight unweighable trace of sulphate.

phate a heavy precipitate; and on saturation with ammonium sulphate in the cold an abundant gummy precipitate, the latter plainly indicating the presence of caseose. As the product originally gave no precipitate with ammonium sulphate, it would apparently follow that the peptone by long drying at 110° C. had been, in part, reconverted into caseose, and as the solution gave no turbidity by heat it would imply that the caseose formed by the reconversion of the peptone was the deutero body. Results similar to these were obtained by Kühne and Chittenden with fibrin peptone.

In a second digestion of trypsin, 1 kilo. of moist casein was warmed at 40° C. with 4 litres of 0·6 per cent. sodium carbonate solution containing trypsin, well thymolized, for one week. On the third day, the gelatinous coagulum already described made its appearance, but gradually disappeared and at the end of the week there was only a very small residue remaining. The neutralized and concentrated digestive fluid, freed from more or less tyrosin by cooling and crystallization, was in this case treated with rock salt to saturation, yielding, however, only a small precipitate of proto- and heterocaseose, which on purification agreed in reactions with the caseoses previously described. Addition of salt-saturated acetic acid to the sodium chloride filtrate from the foregoing caseoses, failed to give any precipitate whatever, and as the saturation of the fluid with ammonium sulphate gave only a slight gummy precipitate it is evident that in this digestion the casein was almost completely converted into peptone. In order to be quite sure of the complete removal of everything precipitable by the ammonium salt the mixture was boiled for some time with an excess of ammonium sulphate, and the filtrate treated as described under peptone *A* for the complete removal of tyrosin and ammonium sulphate.

The final product was exceedingly gummy and parted with the last traces of adhering alcohol very slowly. In fact, we found it best to dissolve the final alcoholic precipitate of peptone in a little water, and to drive off the alcohol from the solution by heat, after which the fluid was evaporated and the gummy peptone finally transformed into a friable mass by drying on a water-bath, and at last completely dried at 110° C. After its final precipitation with alcohol, an aqueous solution of the peptone gave no precipitate whatever, with nitric or acetic acid, neither with acetic acid and potassium ferrocyanide nor with cupric sulphate, or at the most nothing more than a faint turbidity. After being dried at 110° C. until of constant weight, the product then gave a decided gummy precipitate by saturation

of its aqueous solution with ammonium sulphate, and like peptone *A* gave precipitates with nitric and acetic acid and with cupric sulphate.

On analysis the following results were obtained:

*Casein antipeptone B.*

I. 0·3770 gram substance gave 0·2023 gram $H_2O$=5·96 per cent. H and 0·6535 gram $CO_2$=47·25 per cent. C.

II. 0·2687 gram substance gave 0·1442 gram $H_2O$=5·96 per cent. H and 0·4667 gram $CO_2$=47·36 per cent. C.

III. 0·3732 gram substance gave 44·9 c. c. N at 14·4° C. and 757·0 mm pressure=14·31 per cent. N.

IV. 0·3801 gram substance gave 46·0 c. c. N at 14·0° C. and 756·0 mm pressure=14·38 per cent. N.

V. 0·4760 gram substance gave 0·0374 gram ash=7·86 per cent.

VI. 0·5396 gram substance gave 0·0429 gram ash=7·95 per cent.

*Percentage composition of ash-free substance.*

| | | | | | Average. |
|---|---|---|---|---|---|
| C | 51·35 | 51·42 | .... | .... | *51·38* |
| H | 6·47 | 6·47 | .... | .... | *6·47* |
| N | .... | .... | 15·54 | 15·61 | *15·57* |

In a third digestion with trypsin, 2 kilos. of casein were warmed at 40° C. for five days with 3 litres of 0·5 per cent. sodium carbonate containing an active trypsin solution, well thymolized. At the end of the second day, considerable casein antialbumid separated from the solution, this time more as a gummy precipitate than as a gelatinous coagulum. On the sixth day, the alkaline fluid was filtered from the small undigested residue, neutralized, concentrated, and the caseoses precipitated by saturation with ammonium sulphate. In this digestion, there was present only a very small trace of caseose precipitable by saturation with salt, but considerable precipitable by salt-saturated acetic acid. After repeated boiling of the ammonium sulphate-saturated fluid, for complete removal of caseoses, the peptone remaining was separated, purified and dried as already described. The product dried at 110° C. gave on analysis the following results.

*Casein antipeptone C.*

I. 0·3104 gram substance gave 0·1720 gram $H_2O$=6·15 per cent. H and 0·5252 gram $CO_2$=46·14 per cent. C.

II. 0·2756 gram substance gave 0·1513 gram $H_2O$=6·10 per cent. H and 0·4638 gram $CO_2$=45·89 per cent. C.

III. 0·5432 gram substance gave 68·0 c. c. N at 14·6° C. and 756·4 mm pressure=14·88 per cent. N.

IV. 0·6280 gram substance gave 0·0449 gram ash=7·15 per cent.

V. 0·4060 gram substance gave 0·0288 gram ash=7·09 per cent.

*Percentage composition of ash-free substance.*

| | | | | Average. |
|---|---|---|---|---|
| C | 49·64 | 49·40 | ---- | 49·52 |
| H | 6·63 | 6·57 | ---- | 6·60 |
| N | ---- | ---- | 15·99 | 15·99 |

This peptone, like the preceding, after being dried at 110° C. gave a small gummy precipitate on saturation of its solution with ammonium sulphate, and also gave a precipitate with nitric and acetic acid, and a slight turbidity with cupric sulphate.

In composition, all three of the peptones show a smaller percentage of carbon than the caseoses formed by trypsin, but somewhat to our surprise the percentage of carbon is higher than in some of the caseoses formed by pepsin-hydrochloric acid. The nature of the substance, however, which at present affords but little proof that we have to deal with a single body, the extreme difficulty of obtaining it in a condition of dryness suitable for analysis, and the almost utter impossibility of freeing it from adhering inorganic salts, all tend to throw doubt on the analytical data as expressing the composition of pure casein peptone. As already stated, there is unquestionably more or less of a decomposition or change attending the drying of the peptone. So pronounced is the hygroscopic character of these bodies, that when partially dried they will gain weight over strong sulphuric acid, and on being taken from the air bath while drying at 110° C. we often noticed on damp days a hissing noise as if from the rapid absorption of water. Further, as the drying progressed the odor of valerianic acid became quite pronounced and on testing the dried product, it was often found to have an acid reaction so pronounced in many cases as to give a sour taste to the peptone, in addition to the characteristic bitter. Before drying, the peptones were usually found to have a neutral reaction. These facts coupled with the changed behavior of the product towards ammonium sulphate, point to a change in the nature of the peptone, which may well be assumed to affect its composition, and hence we would have our analytical figures taken with some allowance.

We also prepared a peptone, entirely non-precipitable by saturation with ammonium sulphate, by boiling 20 grams of pure deuterocaseose with 500 c. c. of 3 per cent. sulphuric acid for 14 hours. This preparation, after purification, was too small in quantity for analysis, but in reactions it showed close agreement with the antipeptones prior to their long drying at 110° C., viz: non-precipitation by acetic acid and potassium ferrocyanide, by nitric acid, by cupric sulphate, and by saturation with ammonium sulphate both in neutral and in acid solutions.

## VI.—Some Experiments on the Influence of Arsenic and Antimony on Glycogenic Function and Fatty Degeneration of the Liver. By R. H. Chittenden, and J. A. Blake, B.A., Ph.B.

Saikowsky's* oft-quoted experiments on rabbits with antimonic and arsenious acids have made clear that in both arsenical and antimonial poisoning there is pronounced fatty degeneration of the liver, with a lessening of the hepatic glycogen and in some cases even a total disappearance of it. With antimonic acid, Saikowsky found in his original experiments, that one-half to one gram of antimonic acid or other preparation of antimony per day, for fourteen or nineteen days in succession, gave rise to a fatty degeneration embracing the liver, kidneys, and even the heart. This has been verified by the experiments of Grohe and Mosler, who also state that in the duchy of Brunswick the peasantry give to the geese, when producing the famous fatty livers, a certain quantity of the white oxide of antimony every day.† With arsenic, Saikowsky likewise found that when rabbits are poisoned by a small dose so as to live from three to six days, the liver becomes much enlarged and very fatty and the glycogenic function nearly or quite abolished.

It is very evident, therefore, that in large quantities both arsenic and antimony have a special action on tissue changes, particularly on the liver. In the experiments referred to above, the quantities of poison given were quite large and with arsenic, particularly, their administration was soon followed by death. As neither of these substances are ordinarily used in medicine for an acute effect, it seemed to us of interest to study the action of small doses on the tissue changes of the liver, with a view to ascertaining whether non-toxic doses of these two poisons would produce a similar effect. It is ordinarily stated that in poisoning with antimony, phosphorus, and arsenic the nitrogenous products of tissue waste appear in the urine in much larger quantity than normally, owing to the increased decomposition which is going on.‡ Experiments of our own, however, have shown that

* Virchow's Archives, Band xxxiv, p. 78.

† Quoted from H. C. Wood's Therapeutics, p. 161.

‡ Brunton's Pharmacology, Therapeutics and Materia Medica, p. 360.

small repeated doses of antimonious oxide are without influence on the excretion of nitrogen, sulphur, and phosphorus, and that hence when taken in non-toxic doses it has no noticeable action on proteid metabolism.* Without doubt, toxic doses do materially affect the nutrition of the body, but with a dog of 13 kilos. weight the administration of repeated doses of antimonious oxide, to the extent of 17 grains in 13 days, led to no apparent change in the amount of nitrogen etc. excreted, although the presence in the 24 hours' urine of 13–23 milligrams of antimony (Sb) gave evidence of decided absorption.

We have therefore tried a few experiments on rabbits and fowls to see what effect small repeated doses of arsenic and antimony would have on the liver, as indicated by its content of fat, glycogen, and sugar. The experiments were made in pairs, in which one animal of each pair served as a control for comparison, while the other, kept under the same conditions of diet, etc. so far as possible, was fed each day with arsenic or antimony as the case might be. At the end of the period both animals were killed and the livers analyzed. Naturally, the animals of each pair were of the same age, from the same brood, and so far as possible of the same body weight. During the experiment they were kept on a weighed diet of cracked corn, meal, etc., and were confined in suitable cages.

The methods of analysis were as follows: after determining the body weight, the liver was quickly removed, weighed and sampled by chopping, 10 grams or thereabouts were then weighed out accurately, dried on a water bath, ground to a fine powder and extracted in a fat extractor with warm ether until the fat was entirely removed.

For glycogen and sugar, 20–40 grams of the sampled liver were thoroughly extracted with hot water, (continuous extraction with water, frequently renewed, for several days) the extracts united, concentrated to a very small volume and precipitated with a large excess of 95 per cent. alcohol. The precipitate of glycogen, etc., was washed with alcohol, dissolved in a small volume of cold water to 100 c. c., sufficient hydrochloric acid added to make the fluid contain 2 per cent. HCl and heated on a water-bath for 15 hours. After neutralization, the volume was made up to 200 c. c. and the sugar determined in 25 c. c. of the fluid by Allihn's gravimetric method, and the glycogen calculated therefrom. The liver sugar was determined by evaporating the alcoholic fluid from the glycogen precipitate, dissolving the residue in a little water, adding sufficient sulphuric acid to make the mix-

* Chittenden and Blake. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 94.

ture contain 2 per cent. $H_2SO_4$, boiling for two hours to convert the sugar wholly into dextrose, and then, after neutralization of the acid fluid and diluting to 200 c. c., testing its reducing power by Allihn's method.

### *Experiment* I.

### *Action of arsenic on a fowl.*

*Period of dosing.*

| | |
|---|---|
| May 20–24, | 0·1 grain $As_2O_3$ daily. |
| " 25–31, | 0·2 " " " |
| June 1–15, | 0·3 " " " |
| | 6·4 grains. |

| | *A, control fowl.* | *B, arsenic fowl.* |
|---|---|---|
| Body weight May 20, | 1814 grams. | 1814 grams. |
| " " June 16, | 1871 " | 1686 " |
| | +57 | −128 |
| Weight of liver, June 16, | 28·378 grams. | 34·635 grams. |

*The liver of B, showed unmistakeable signs of fatty degeneration.*

*Determination of fat in liver.*

| | | | |
|---|---|---|---|
| *A.* control, | 9·210 grams liver gave 0·3347 gram fat, | = | 3·63 per cent. |
| *B.* arsenic, | 13·848 " " " 1·8561 " " | = | 13·40 " |
| | | | +9·77 per cent. |

*Determination of glycogen and sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 20·167 grams. | 20·787 grams. |

*Glycogen A, control.*

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·0744 gram. | 0·0380 gram. | 0·0342 gram. | 0·2736 gram. | 1·35 |
| 25 | 0·0727 | 0·0371 | 0·0334 | 0·2672 | 1·32 |

*Glycogen B, arsenic.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·1720 gram. | 0·0879 gram. | 0·0791 gram. | 0·6328 gram. | 3·04 |
| 25 | 0·1713 | 0·0875 | 0·0787 | 0·6296 | 3·02 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0299 gram. | 0·0159 gram. | ----- | 0·1272 gram. | 0·63 |
| 25 | 0·0332 | 0·0176 | ----- | 0·1408 | 0·69 |

*Sugar B, arsenic.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0185 gram. | 0·0102 gram. | ----- | 0·0816 gram. | 0·39 |
| 25 | 0·0162 | 0·0091 | ----- | 0·0728 | 0·35 |

100 grams of breast muscle from *B* gave 0·4 milligram of *As*.

In this experiment, then, we have as the apparent result of the long-continued feeding of arsenic a loss of body weight, a decided increase

in the weight of the liver, a large increase in the liver fat, a gain in the amount of liver glycogen, and a loss in liver sugar.

### *Experiment* II.

### *Action of arsenic on a fowl.*

*Period of dosing.*

| | |
|---|---|
| May 20–24, | 0·1 grain $As_2O_3$ daily. |
| " 25–31, | 0·2 " " " |
| June 1–15, | 0·3 " " " |
| " 16–18, | 0·4 " " " |
| | 7·6 grains. |

Towards the end of the experiment the arsenic fowl appeared to be suffering somewhat from the toxic action of the poison, consequently the arsenic was discontinued after the 18th, but the fowl died on the 21st, and was immediately analyzed.

| | *A, control fowl.* | *B, arsenic fowl.* |
|---|---|---|
| Body weight May 20, | 1644 grams. | 1531 grams. |
| " " June 21, | 1757 " | 1247 " |
| | +113 | −284 |
| Weight of liver, June 21, | 55·830 grams. | 33·729 grams. |

*Determination of fat in liver.*

| | | | |
|---|---|---|---|
| *A*, control, | 13·740 grams liver gave 0·7462 gram fat, | = | 5·40 per cent. |
| *B*, arsenic, | 10·179 " " " 0·4925 " " | = | 4·83 " |
| | | | −0·57 per cent. |

*Determination of glycogen and sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 42·090 grams. | 23·550 grams. |

*Glycogen A, control.*

| Volume used. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·5213 gram. | 0·2500 gram. | 0·2250 gram. | 1·8000 gram. | 4·27 |
| 25 | 0·5228 | 0·2504 | 0·2253 | 1·8024 | 4·28 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0359 gram. | 0·0188 gram. | ..... | 0·1504 gram. | 0·35 |
| 25 | 0·0379 | 0·0198 | ..... | 0·1584 | 0·37 |

In *B*, no trace of a reduction could be obtained for glycogen and only an unweighable trace for sugar.

In this experiment, therefore, we find a decided loss in body weight under the influence of the arsenic, a loss in the weight of the liver, a slight diminution in the liver fat and nearly a complete disappearance of both glycogen and sugar.

## *Experiment* III.

### *Action of arsenic on a rabbit.*

*Period of dosing.*

| | |
|---|---|
| May 25–31, | 0·2 grain $As_2O_3$ daily. |
| June 1–15, | 0·3 " " " |
| " 16–23, | 0·4 " " " |
| | 9·1 grains. |

| | *A, control rabbit.* | *B, arsenic rabbit.* |
|---|---|---|
| Body weight May 25, | 1644 grams. | 1502 grams. |
| " " June 24, | 1616 " | 1586 " |
| | −28 | +84 |
| Weight of liver June 24, | 61·360 grams. | 46·760 grams. |

*Determination of fat in liver.*

| | | | |
|---|---|---|---|
| *A*, control, | 15·320 grams liver gave 0·4126 gram fat, | = | 2·69 per cent. |
| *B*, arsenic, | 14·435 " " " 0·2888 " " | = | 2·00 " |
| | | | −0·69 per cent. |

*Determination of glycogen and sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 45·940 grams. | 32·325 grams. |

*Glycogen A,* control.*

| Volume used for reduction. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·2036 gram. | 0·1045 gram. | 0·0940 gram. | 1·8800 grams. | 4·13 |
| 25 | 0·2027 | 0·1041 | 0·0937 | 1·8740 | 4·07 |

*Glycogen B, arsenic.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·5230 gram. | 0·2503 gram. | 0·2252 gram. | 1·8016 grams. | 5·57 |
| 25 | 0·5223 | 0·2500 | 0·2250 | 1·8000 | 5·54 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0177 gram. | 0·0098 gram. | ..... | 0·0784 gram. | 0·17 |
| 25 | 0·0173 | 0·0097 | ..... | 0·0776 | 0·16 |

*Sugar B, arsenic.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0254 gram. | 0·0137 gram. | ..... | 0·1096 gram. | 0·34 |
| 25 | 0·0232 | 0·0126 | ..... | 0·1008 | 0·31 |

100 grams of muscle tissue yielded 0·3 milligram of *As*.

Here, we find under the influence of the arsenic, an apparent gain, though slight, in body weight, an evident diminution in the weight of the liver, together with a diminished percentage of liver fat. Apparently, however, the amount of liver glycogen and sugar are somewhat increased. The liver showed no approach to fatty degeneration on microscopic examination.

* The glycogen solution after boiling with acid, etc., was made up to 500 c. c. instead of 200 c. c.

## *Experiment* IV.

### *Action of antimony on a fowl.*

*Period of dosing.*

| | |
|---|---|
| May 20–24, | 0·2 grain $Sb_2O_3$ daily. |
| " 25–31, | 0·4 " " " |
| June 1–7, | 0·6 " " " |
| " 8–14, | 0·8 " " " |
| | 13·6 grains. |

| | *A, control fowl.* | *B, antimony fowl.* |
|---|---|---|
| Body weight May 20, | 1559 grams, | 1616 grams, |
| " " June 15, | 1531 " | 1828 " |
| | −28 | +212 |
| Weight of liver June 15, | 22·995 grams. | 31·799 grams. |

*Determination of fat in liver.*

| | | | |
|---|---|---|---|
| *A*, control, | 7·985 grams liver gave 0·4600 gram fat, | = | 5·76 per cent. |
| *B*, antimony, | 11·409 " " " 0·6741 " " | = | 5·91 " |
| | | | +0·15 per cent. |

*Determination of glycogen and Sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 15·010 grams. | 20·390 grams. |

*Glycogen A, control.*

| Volume used for reduction. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·0577 gram. | 0·0296 gram. | 0·0266 gram. | 0·2128 gram. | 1·41 |
| 25 | 0·0563 | 0·0289 | 0·0260 | 0·2080 | 1·38 |

*Glycogen B, antimony.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·1011 gram. | 0·0514 gram. | 0·0462 gram. | 0·3696 gram. | 1·81 |
| 25 | 0·1015 | 0·0516 | 0·0464 | 0·3712 | 1·82 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0198 gram. | 0·0109 gram. | ----- | 0·0872 gram. | 0·58 |
| 25 | 0·0211 | 0·0115 | ----- | 0·0920 | 0·61 |

*Sugar B, antimony.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0300 gram. | 0·0160 gram. | ----- | 0·1280 gram. | 0·62 |
| 25 | 0·0277 | 0·0148 | ----- | 0·1184 | 0·58 |

100 grams of breast muscle gave 1·2 milligrams Sb.

These results would seem to indicate that antimonious oxide tends to increase the body weight, and apparently also that of the liver. The liver fat is obviously not much affected in this experiment, while the glycogen shows a slight increase in the antimonial fowl.

## *Experiment* V.

### *Action of antimony on a fowl.*

*Period of dosing.*

| | |
|---|---|
| May 20–24, | 0·2 grain $Sb_2O_3$ daily. |
| " 25–31, | 0·4 " " " |
| June 1– 7, | 0·6 " " " |
| " 8–15, | 0·8 " " " |
| " 16–19, | 1·0 " " " |
| | 18·4 grains. |

| | *A, control fowl.* | *B, antimony fowl.* |
|---|---|---|
| Body weight May 20, | 1701 grams. | 1757 grams. |
| " " June 20, | 1644 " | 1814 " |
| | −57 | +57 |
| Weight of liver June 20, | 34·177 grams. | 35·440 grams. |

*Determination of fat in liver.*

| | | | | |
|---|---|---|---|---|
| *A*, control, | 9·477 grams liver gave | 0·3890 gram fat, | = | 4·10 per cent. |
| *B*, antimony, | 9·579 " " " | 0·5088 " " | = | 5·31 " |
| | | | | +1·21 per cent. |

The appearance of the liver in *B* indicated fatty degeneration.

*Determination of glycogen and sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 24·700 grams. | 25·871 grams. |

*Glycogen A, control.*

| Volume used for reduction. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·2253 gram. | 0·1160 gram. | 0·1044 gram. | 0·8352 gram. | 3·38 |
| 25 | 0·2253 | 0·1160 | 0·1044 | 0·8352 | 3·38 |

*Glycogen B, antimony.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·2480 gram. | 0·1281 gram. | 0·1153 gram. | 0·9224 gram. | 3·56 |
| 25 | 0·2479 | 0·1280 | 0·1150 | 0·9200 | 3·55 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0360 gram. | 0·0189 gram. | ----- | 0·1512 gram. | 0·61 |
| 25 | 0·0360 | 0·0189 | ----- | 0·1512 | 0·61 |

*Sugar B, antimony.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0383 gram. | 0·0200 gram. | ----- | 0·1600 gram. | 0·62 |
| 25 | 0·0372 | 0·0195 | ----- | 0·1560 | 0·62 |

100 grams of breast muscle gave 1·3 milligrams Sb.

**In this experiment, there was pronounced fatty degeneration of the liver in the antimonial fowl. Further, as in the last experiment, there was apparently a slight increase both in body weight and in the weight of the liver.**

Corresponding with the fatty degeneration, there was found 1·2 per cent. more fat in the antimonial liver than in the control.

The liver glycogen was also slightly increased under the influence of the antimony.

## *Experiment* VI.

### *Action of antimony on a rabbit.*

*Period of dosing.*

| | |
|---|---|
| May 25–31, | 0·4 grain $Sb_2O_3$ daily. |
| June 1– 7, | 0·6 " " " |
| " 8–15, | 0·8 " " " |
| " 16–21, | 1·0 " " " |
| | 19·4 grains. |

| | *A, control rabbit.* | *B,* antimony rabbit.* |
|---|---|---|
| Body weight May 25, | 2012 grams. | 1814 grams. |
| " " June 23, | 1998 " | 1701 " |
| | —14 | —113 |
| Weight of liver June 23, | 38·219 grams. | 42·741 grams. |

*Determination of fat in liver.*

| | | | |
|---|---|---|---|
| *A*, control, | 9·375 grams liver gave 0·3575 gram fat, | = | 3·81 per cent. |
| *B*, antimony, | 10·971 " " " 0·3432 " " | = | 3·12 " |
| | | | —0·69 per cent. |

*Determination of glycogen and sugar.*

| | *A.* | *B.* |
|---|---|---|
| Weight of liver used, | 28·844 grams. | 31·770 grams. |

*Glycogen A, control.*

| Volume used for reduction. | Weight Cu. | Equivalent in dextrose. | Equivalent in glycogen. | Total amount. | Per cent. |
|---|---|---|---|---|---|
| 25 c. c. | 0·0431 gram. | 0·0224 gram. | 0·0201 gram. | 0·1608 gram. | 0·55 |
| 25 | 0·0441 | 0·0229 | 0·0206 | 0·1648 | 0·57 |

*Sugar A, control.*

| | | | | | |
|---|---|---|---|---|---|
| 25 c. c. | 0·0445 gram. | 0·0231 gram. | ----- | 0·1848 gram. | 0·64 |
| 25 | 0·0440 | 0·0229 | ----- | 0·1832 | 0·63 |

In *B*, there was no reduction for either glycogen or sugar.

100 grams of muscle gave 1·0 milligram Sb.

In this experiment, towards the end, the animal was evidently suffering from the toxic action of the antimony, and the body weight is seen to be noticeably diminished. The weight of the liver, however, was apparently increased by the antimony, although there was a

* The last two days the antimony rabbit ate very little. Hence, the oxide was not given after the 21st. The animal was evidently much affected by the antimony and its general nutrition was at the last very poor.

diminution in the liver fat, and a complete disappearance of both glycogen and sugar.

While these experiments are far too few in number to generalize from, yet it would appear that small doses of antimonious oxide long continued tend to increase body weight, and particularly the weight of the liver. Further, the increase in liver weight is accompanied by an increased percentage of fat and a slight increase of liver glycogen.

With corresponding doses of arsenious oxide, on the other hand, the results would indicate a diminution in body weight, likewise a diminution in the weight of the liver and also in the amount of liver fat. As regards glycogen, two of the results show an increased amount in the arsenical livers, while in one experiment there was a total disappearance of glycogen. On the other hand, as Experiment No. I indicates, there may be, with small doses of arsenic, a very pronounced fatty degeneration of the liver, accompanied by an increased liver weight and an increase in the percentage of liver glycogen.

## VII.—The Nature and Chemical Composition of the Myosin of Muscle Tissue. By R. H. Chittenden and G. Wyckoff Cummins, Ph.D.

In spite of the interest attached to this peculiar proteid substance, little attention has been directed to its chemical nature since the time of its discovery by Kühne.* In fact, Danilewsky† is the only investigator who appears to have studied its chemical relations to any extent, and so far as we are aware no attempt has ever been made to ascertain its chemical composition. With this fact in mind, it has been the main object of the present investigation to study the chemical composition of pure myosin, and to determine the differences that may exist in the chemical nature, or in the properties, of myosin prepared from various animal sources. Unfortunately, our work was completed before Halliburton's‡ recent paper on muscle-plasma was published, otherwise we should have attempted to verify some of his interesting discoveries regarding the muscle clot, and possibly have modified somewhat the character of our work.

The researches of Kühne, Hoppe-Seyler,§ Weyl,‖ and Danilewsky have shown that myosin, both from the animal and vegetable kingdoms, is a globulin body, soluble in dilute sodium and ammonium chloride solutions, as well as in sulphate of magnesia and sulphates of the alkalies, and precipitable therefrom either by the addition of salt in substance or by dilution with much water. As stated by Danilewsky, myosin is most advantageously extracted from muscle tissue by ammonium chloride, in solutions of from 7–20 per cent. We have, likewise, found this to be the case by comparative tests, and have, therefore, in all of our preparations of myosin for analysis, used ammonium chloride as the extractive, preferably of 15 per cent. strength. For separation of myosin from the ammonium chloride

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* Kühne, Protoplasma, Leipzig, 1864. Also Lehrbuch der physiologische Chemie.

† Myosin, seine Darstellung, Eigenschaften, Umwandlung in Syntonin und Rückbildung aus demselben. Zeitschrift für physiologische chemie. Band v, p. 158.

‡ Journal of Physiology, vol. viii, p. 133.

§ Handbuch der Chem. Analyse, 4 Auflage, p. 236.

‖ Beitrage zur kenntniss thierischer und pflanzlicher eiweisskörper, Zeitschrift für physiologische Chemie, Band i, p. 72.

solution we have mainly employed two methods, either dilution with a large volume of distilled water, or dialysis of the ammonium chloride solution until the salt is entirely removed. In this latter process, we have often observed that the separation of myosin partakes more of the nature of a coagulation than of an ordinary precipitation. A moderately strong ammonium chloride solution of myosin, on being placed in a parchment bag and suspended in running water, will ordinarily at the end of two or three days be converted into a semi-solid, jelly-like mass, which later on contracts more or less, but still shows all the characters of a genuine clot. In fact, we are inclined, with Halliburton, to consider this a genuine re-coagulation rather than a precipitation. We are inclined, however, to believe that separation of myosin by dialysis is hardly as satisfactory in the preparation of small quantities, as precipitation by water, since on dialysis the jellying of the myosin naturally tends to enclose some of the salt and also any other proteids possibly present in the solution, while by precipitation with water the myosin is flocculent, easily washed and thus more surely freed from both salt and albumin.

*Myosin A, from ox muscle.*

The first sample of myosin was prepared from a freshly killed ox. The finely chopped muscle from the thigh was freed, so far as possible, from all traces of blood and soluble albumin by long soaking and frequent kneading with water. The water was frequently changed and kept thoroughly thymolized so as to prevent any approach to putrefaction. When the washings failed to give any turbidity by heat, or by acetic acid and potassium ferrocyanide, the washed tissue was placed in 10 litres of a 15 per cent. solution of ammonium chloride for extraction of the myosin. The resultant opalescent fluid was filtered through paper and the myosin precipitated by saturation with sodium chloride. The precipitate was quickly strained off, dried somewhat between folds of filter paper, dissolved in a small amount of water and reprecipitated by treatment of the fluid with a large volume of water. The precipitate so obtained was washed with water until the washings gave no reaction for chlorides, when it was treated with weak alcohol and finally with 95 per cent. and absolute alcohol, and ether.

When partially dried, it was ground fine and further dried at 110° C. until of constant weight, for analysis.

Its composition is shown in the accompanying table.

ANALYSIS OF MYOSIN A, FROM OX MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3878 | 0·2442 | 6·99 | 0·7400 | 52·04 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·5659 | 0·3579 | 7·03 | 1·0809 | 52·09 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·5996 | ------ | --- | ------ | ---- | 81·7 | 8·7 | 758·4 | 16·61 | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·5783 | ------ | --- | ------ | ---- | 80·1 | 10·0 | 757·7 | 16·69 | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·6794 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0101 | 1·49 | ---- | --- | ---- | --- | ----- |
| VI | 0·8173 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0121 | 1·48 | ---- | --- | ---- | --- | ----- |
| VII | 1·4967 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0064 | 0·05 | ---- | --- | ----- |
| VIII | 0·6930 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0799 | 1·58 | 1·53 |
| IX | 0·9133 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0965 | 1·44 | 1·39 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·83 | 52·84 | ---- | ---- | ---- | ---- | *52·84* |
| H | 7·10 | 7·13 | ---- | ---- | ---- | ---- | *7·12* |
| N | ---- | ---- | 16·84 | 16·94 | ---- | ---- | *16·89* |
| S | ---- | ---- | ---- | ---- | 1·56 | 1·42 | *1·49* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *21·66* |
| | | | | | | | *100·00* |

### *Myosin B, from ox muscle.*

A solution of myosin in 15 per cent. ammonium chloride was prepared from 4 kilos. of freshly killed beef, as described under A. The myosin was then separated from the solution by dialysis, continued until nearly all of the chloride was removed. The last traces of the ammonium salt were separated by filtration of the gelatinous myosin through chamois skin, and washing with water. The preparation was then treated exactly as A, and dried at 110° C. for analysis.

By long continued contact with water, as in dialysis, the precipitated or coagulated myosin is rendered insoluble in dilute salt solutions, as noticed by Weyl and others, hence in this preparation it was not possible to purify the substance by reprecipitation.

The composition of the product (see the accompanying table) is essentially the same as that of the preceding preparation.

### *Myosin C, from sheep's muscle.*

This sample of myosin was obtained from fresh mutton in essentially the same manner as the preceding preparation, viz: by extraction of the thoroughly washed tissue with 15 per cent. ammonium chloride, and separation of the myosin by dialysis.

On analysis, it was found to possess a somewhat higher percentage of carbon than the two myosins from ox muscle, but in other respects it was identical with them.

### *Myosin D, from calf's muscle.*

A solution of myosin was prepared from 3 kilos. of fresh, lean muscle from a young calf, by extraction of the thoroughly washed tissue with 15 per cent. ammonium chloride solution.

From one-half of the filtered fluid, myosin was precipitated by dilution with water and purified by washing with water until chlorides were entirely removed. It was then washed with weak alcohol, finally with absolute alcohol and ether, and then dried at 110° C. for analysis ($D^1$).

From the other half of the ammonium chloride solution, myosin was separated by addition of ammonium chloride in substance. The precipitate was freed from excess of ammonium chloride by addition of just enough water to dissolve the salt, after which the flocculent myosin was strained off, dissolved in a little water, and precipitated by pouring the solution into a large volume of water. It was

ANALYSIS OF MYOSIN B, FROM OX MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4306 | 0·2719 | 7·01 | 0·8287 | 52·16 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·3932 | 0·2500 | 7·06 | 0·7515 | 52·12 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0.5061 | ------ | --- | ------ | ---- | 67·8 | 9·8 | 764·6 | 16·34 | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·6005 | ------ | --- | ------ | ---- | 81·3 | 9·7 | 762·6 | 16·48 | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 1·0425 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0072 | 0·69 | ---- | --- | ---- | --- | ----- |
| VI | 0·6690 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0046 | 0·69 | ---- | --- | ---- | --- | ----- |
| VII | 1·7115 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0046 | 0·05 | ---- | --- | ----- |
| VIII | 0·6277 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0597 | 1·31 | 1·26 |
| IX | 0·6358 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0594 | 1·29 | 1·24 |

| | *Percentage composition of the ash-free substance.* | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·53 | 52·48 | --- | ---- | ---- | ---- | *52·51* |
| H | 7·06 | 7·11 | ---- | ---- | ---- | ---- | *7·09* |
| N | ---- | ---- | 16·46 | 16·59 | ---- | ---- | *16·53* |
| S | ---- | ---- | ---- | ---- | 1·26 | 1·24 | *1·25* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·62* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN C, FROM SHEEP'S MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T.°C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4945 | 0·3150 | 7·08 | 0·9603 | 52·95 | --- | --- | ---- | --- | ---- | --- | --- | --- | ---- | --- | ----- |
| II | 0·3539 | 0·2255 | 7·08 | 0·6863 | 52·88 | --- | --- | ---- | --- | ---- | --- | --- | --- | ---- | --- | ----- |
| III | 0·4600 | ---- | ---- | ---- | ---- | 61·8 | 10·2 | 761·1 | 16·29 | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·7477 | ---- | ---- | ---- | ---- | 101·3 | 10·2 | 762·1 | 16·41 | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·5170 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0031 | 0·60 | ---- | --- | ---- | --- | ----- |
| VI | 0·8969 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0051 | 0·57 | ---- | --- | ---- | --- | ----- |
| VII | 1·4139 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0037 | 0·036 | ---- | --- | ----- |
| VIII | 0·7761 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0699 | 1·24 | 1·20 |
| IX | 0·7059 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0667 | 1·29 | 1·25 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·27 | 53·20 | ---- | ---- | ---- | ---- | *53·24* |
| H | 7·12 | 7·12 | ---- | ---- | ---- | ---- | *7·12* |
| N | ---- | ---- | 16·39 | 16·51 | ---- | ---- | *16·45* |
| S | ---- | ---- | ---- | ---- | 1·20 | 1·25 | *1·23* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *21·96* |
| | | | | | | | *100·00* |

then washed with water, alcohol, and ether, and dried at 110° C. ($D^{II}$).

The composition of both products is shown in the accompanying tables.

From the analytical data it is seen that the two preparations show close agreement, although there are minor differences; $D^{II}$, for example, containing a slightly higher percentage of carbon and a correspondingly lower percentage of nitrogen than $D^{I}$. Further, $D^{II}$ contains less than half as much ash as the other preparation. Both show fairly close agreement with the myosins from ox muscle, with perhaps a slightly higher average percentage of nitrogen.

### *Myosin E, from fish.*

So far as we are aware, little attention has been paid to the myosin from fish flesh. Myosin is assumed to be present and is supposed to be of the same general nature as the myosin from other forms of muscle tissue.

We first tried the separation of myosin from fresh cod (*Gadus callarias* or *G. morrhua*), using 2 kilos. of the fresh, lean tissue obtained in market. The final ammonium chloride extract (15 per cent.) failed to give anything more than an insignificant precipitate, either by dilution with water or by saturation of the fluid with sodium chloride.

A second preparation was attempted from the flesh of the halibut (*Hippoglossus vulgaris*); 2·5 kilos. of fresh tissue, free from fasciæ, fat and integument were thoroughly extracted with thymolized water for several days, the chopped tissue being well rubbed up with the water to insure complete removal of soluble albumins, etc. The thoroughly washed fibre was then triturated with a 15 per cent. ammonium chloride solution and allowed to stand in contact with it for 24 hours. The filtered fluid gave a decided precipitate on boiling, and also on addition of salt to saturation.

Myosin was separated from the ammonium chloride solution by dialysis as a more or less gelatinous precipitate, and was prepared for analysis by washing with thymolized water, and treatment with alcohol and ether.

Dried at 110° C. until of constant weight, it yielded the following results:

I. 0·3335 gram substance gave 0·2043 gram $H_2O$ = 6 08 per cent. H and 0·6257 gram $CO_2$ = 51·16 per cent. C.

**Analysis of Myosin D[1], from calf's muscle.**

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3541 | 0·2201 | 6·91 | 0·6646 | 51·18 | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·3925 | 0·2439 | 6·93 | 0·7395 | 51·33 | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·6283 | ---- | ---- | ---- | ---- | 87·4 | 9·8 | 749·8 | 16·64 | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·6065 | ---- | ---- | ---- | ---- | 82·6 | 10·7 | 768·1 | 16·64 | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0·9821 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0291 | 2·95 | ---- | --- | --- | --- | ----- |
| VI | 0·7361 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | 0·0214 | 2·91 | ---- | --- | ---- | --- | ----- |
| VII | 1·7182 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | 0·0130 | 0·10 | ---- | --- | ----- |
| VIII | 0·6707 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0679 | 1·39 | 1·29 |
| IX | 0·5690 | ---- | ---- | ---- | ---- | --- | --- | ---- | --- | ---- | --- | ---- | --- | 0·0575 | 1·39 | 1·29 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·79 | 52·88 | ---- | ---- | ---- | ---- | *52·84* |
| H | 7·11 | 7·11 | ---- | ---- | ---- | ---- | *7·11* |
| N | ---- | ---- | 17·14 | 17·14 | ---- | ---- | *17·14* |
| S | ---- | ---- | --- | ---- | 1·88 | 1·88 | *1·53* |
| O | ---- | --- | --- | ---- | ---- | ---- | *21·68* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN $D^{II}$, FROM CALF'S MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$, gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3284 | 0·2078 | 7·03 | 0·6803 | 52·34 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| II | 0·2290 | 0·1457 | 7·07 | 0·4396 | 52·35 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| III | 0·6012 | ------ | --- | ------ | ---- | 82·8 | 11·0 | 768·1 | 16·81 | ---- | --- | ---- | --- | ---- | --- | ---- |
| IV | 0·5682 | ------ | --- | ------ | ---- | 78·5 | 11·4 | 762·5 | 16·71 | ---- | --- | ---- | --- | ---- | --- | ---- |
| V | 0·5994 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0070 | 1·17 | ---- | --- | ---- | --- | ---- |
| VI | 0·8031 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | 0·0096 | 1·19 | ---- | --- | ---- | --- | ---- |
| VII | 1·4025 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | 0·0037 | 0·036 | ---- | --- | ---- |
| VIII | 0·5575 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0521 | 1·28 | 1·24 |
| IX | 0·6235 | ------ | --- | ------ | ---- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0576 | 1·27 | 1·23 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·97 | 52·97 | ---- | ---- | ---- | ---- | *52·97* |
| H | 7·11 | 7·15 | ---- | ---- | ---- | ---- | *7·13* |
| N | ---- | ---- | 17·01 | 16·91 | ---- | ---- | *16·96* |
| S | ---- | ---- | ---- | ---- | 1·26 | 1·24 | *1·25* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *21·69* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN F[1], FROM OX MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4229 | 0·2661 | 6·99 | 0·8109 | 52·29 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·3655 | 0·2315 | 7·03 | 0·7019 | 52·36 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·6159 | ------ | --- | ------ | --- | 84·3 | 10·1 | 754·3 | 16·45 | ---- | -- | ---- | --- | ---- | --- | ----- |
| IV | 0·6100 | ------ | --- | ------ | --- | 83·6 | 9·2 | 755·7 | 16·57 | ---- | --- | --- | --- | ---- | --- | ----- |
| V | 0·7144 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0088 | 1·23 | ---- | --- | ---- | --- | ----- |
| VI | 0·7000 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0090 | 1·29 | ---- | --- | ---- | --- | ----- |
| VII | 1·4144 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | 0·0050 | 0·05 | ---- | --- | ----- |
| VIII | 0·6112 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0579 | 1·30 | 1·25 |
| IX | 0·5189 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | --- | --- | 0·0529 | 1·40 | 1·35 |
| X | 0·5882 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0545 | 1·27 | 1·22 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|
| C | 52·95 | 53·04 | ---- | ---- | ---- | ---- | ---- | *52·99* |
| H | 7·08 | 7·13 | ---- | ---- | ---- | ---- | ---- | *7·11* |
| N | ---- | ---- | 16·67 | 16·78 | ---- | ---- | ---- | *16·73* |
| S | ---- | ---- | ---- | ---- | 1·27 | 1·37 | 1·24 | *1·29* |
| O | - | | --- | --- | -- | -- | -- | *21·88* |
| | | | | | | | | *100·00* |

ANALYSIS OF MYOSIN F$^{II}$, FROM OX MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3369 | 0·2113 | 7·00 | 0·6381 | 51·63 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| II | 0·4515 | 0·2804 | 6·92 | 0·8582 | 51·83 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ----- |
| III | 0·6228 | ------ | --- | ------ | --- | 84·4 | 10·8 | 758·0 | 16·33 | ---- | --- | ---- | --- | ---- | --- | ----- |
| IV | 0·4050 | ------ | --- | ------ | --- | 55·5 | 10·1 | 754·2 | 16·48 | ---- | --- | ---- | --- | ---- | --- | ----- |
| V | 0.5905 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0123 | 2·08 | ---- | --- | ---- | --- | ----- |
| VI | 0·7229 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0146 | 2·02 | ---- | --- | ---- | --- | ----- |
| VII | 1·3134 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | 0·0074 | 0·077 | ---- | --- | ----- |
| VIII | 0·5634 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0565 | 1·38 | 1·30 |
| IX | 0·7243 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0705 | 1·32 | 1·24 |

*Precentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·73 | 52·92 | ---- | ---- | ---- | --- | *52·83* |
| H | 7·14 | 7·05 | ---- | ---- | ---- | ---- | *7·10* |
| N | ---- | ---- | 16·67 | 16·81 | ---- | ---- | *16·74* |
| S | ---- | ---- | ---- | ---- | 1·33 | 1·28 | *1·31* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·02* |
| | | | | | | | *100·00* |

II. 0·4895 gram substance gave 67·8$^{cc}$ N at 15·4° C. and 761·6$^{mm}$ pressure = 16·50 per cent. N.

III. 0·4939 gram substance gave 0·0116 gram ash = 2·35 per cent.

Percentage composition of ash-free substance,

C 52·39, H 6·97, N 16·74.

The amount of myosin obtained from the fish muscle was not at all commensurate with the amount of flesh experimented with. Danilewsky,* however, found in the muscle tissue of the perch 8·56 per cent. of myosin as contrasted with 7·5 per cent. in ox muscle. Whether our inability to separate a corresponding amount from the tissue of the cod and halibut was due to the character of the tissue, to its possible staleness, or to inferior methods of separation we cannot say. Certainly, the yield of myosin in our case was not as great as from the muscle tissue of the ox, calf, lamb or sheep.

In composition, and in such reactions as we were able to try, the fish myosin was not unlike the myosin from other preparations.

### *Myosin F, from ox muscle.*

A solution in 15 per cent. ammonium chloride was prepared as in preceding cases and the myosin precipitated by addition of a large amount of water.

One half of the precipitated myosin was washed with water until all ammonium chloride was removed, then with alcohol and ether, and dried for analysis ($F^{I}$).

The other half of the myosin was washed somewhat with water, not enough to render it insoluble, then dissolved in 10 per cent. sodium chloride solution, reprecipitated by dilution with water, thoroughly washed and dried ($F^{II}$).

The composition of the two products is shown in the accompanying tables. The results show close agreement with each other, and with the preceding preparations.

### *Myosin G, from ox muscle.*

According to Danilewsky, a 15 per cent. solution of ammonium chloride is stronger than necessary for the extraction of myosin from

* Ueber die Abhänghigkeit der contractionsart der muskeln von der mengen verhältnissen einiger ihrer Bestandtheile. Zeitschrift für physiologische Chemie. Band vii, p. 124.

muscle tissue. In fact, microscopic examination showed that 15 per cent. solutions of this salt generally cause destruction of the muscle fibres, while weaker solutions of the ammonium salt, as 5 per cent., extract the myosin equally well, without injuring the structural elements in the least.

Thinking that possibly a purer myosin might be obtained by the use of a weaker salt solution, several kilos. of thoroughly washed muscle tissue were rubbed up with an excess of 5 per cent. ammonium chloride and kept in contact with it for 48 hours. From the filtered solution, myosin was separated by addition of a large volume of water and the product washed and dried for analysis ($G^{I}$).

The tissue remaining after extraction as described, was treated with a fresh 5 per cent. solution of ammonium chloride, and from this fluid a second precipitate of myosin was obtained by addition of water. This also was washed and dried for analysis ($G^{II}$).

The residue of tissue still remaining was then extracted with a 15 per cent. solution of ammonium chloride. The extract so obtained gave a flocculent precipitate on saturation with sodium chloride, and a coagulum appeared on boiling the solution, but no precipitate could be obtained on addition of a large volume of water. It is thus evident that there was an almost complete extraction of myosin by the 5 per cent. solution of the ammonium salt.

On precipitating the small amount of myosin present in the 15 per cent. ammonium chloride solution by saturation of the fluid with salt, a clear filtrate was obtained which gave a turbidity on boiling and also on addition of acetic acid and potassium ferrocyanide, thus showing the presence of a small amount of soluble albumin.

The composition of the two myosins, as seen from the accompanying tables, does not differ at all from that of other preparations.

### *Myosin H, from muscle of sheep.*

In this preparation, the thoroughly washed and chopped muscle was partially extracted with a 5 per cent. ammonium chloride solution, and the myosin separated by addition of water. It was then washed and dried for analysis ($H^{I}$).

The residue of tissue was extracted with a 15 per cent. solution of ammonium chloride and the myosin separated from this solution, likewise, by addition of water ($H^{II}$).

ANALYSIS OF MYOSIN G[1], FROM OX MUSCLE.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S %. | S after deducting S of ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3877 | 0·2482 | 7·11 | 0·7437 | 52·31 | ... | ... | ..... | ... | .... | ... | .... | ... | .... | ... | ..... |
| II | 0·4690 | 0·2974 | 7·05 | 0·8979 | 52·21 | ... | ... | ..... | ... | .... | ... | .... | ... | .... | ... | ..... |
| III | 0·6343 | ..... | ... | ..... | ... | 85·5 | 12·0 | 761·8 | 16·24 | .... | ... | .... | ... | ... | ... | ..... |
| IV | 0.5718 | ..... | ... | ..... | ... | 77·6 | 13·6 | 765·6 | 16·32 | .... | ... | .... | ... | .... | ... | ..... |
| V | 0·4814 | ..... | ... | ..... | ... | ... | ... | ..... | ... | 0·0071 | 1·47 | .... | ... | .... | ... | ..... |
| VI | 0·7234 | ..... | ... | ..... | ... | ... | ... | ..... | ... | 0·0109 | 1·51 | .... | ... | .... | ... | ..... |
| VII | 1·2048 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | 0·0071 | 0·08 | .... | ... | ..... |
| VIII | 0·5281 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | .... | ... | 0·0484 | 1·26 | 1·18 |
| IX | 0·5719 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | .... | ... | 0·0506 | 1·22 | 1·14 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 53·09 | 53·00 | .... | .... | .... | .... | *53·05* |
| H | 7·22 | 7·16 | .... | .... | .... | .... | *7·19* |
| N | .... | .... | 16·47 | 16·57 | .... | .... | *16·52* |
| S | .... | .... | .... | .... | 1·20 | 1·16 | *1·18* |
| O | .... | .... | .... | .... | .... | | *22·06* |
| | | | | | | | *100·00* |

[illegible] OF MYOSIN [illegible], FROM OX MUSCLE.

| No. | Substance used. gram | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4788 | 0·3021 | 7·01 | 0·9077 | 51·68 | ... | ... | ..... | ... | .... | ... | ... | ... | .... | .... | ..... |
| II | 0·3802 | 0·2361 | 6·90 | 0·7221 | 51·78 | ... | ... | ..... | ... | .... | ... | .... | ... | .... | .... | ..... |
| III | 0·8286 | ..... | ... | ..... | ... | 113·3 | 13·6 | 765·8 | 16·43 | .... | ... | .... | ... | .... | .... | ..... |
| IV | 0·4869 | ..... | ... | ..... | ... | 66·5 | 14·1 | 768·4 | 16·49 | .... | ... | .... | ... | .... | .... | ..... |
| V | 0·5740 | ..... | ... | ..... | ... | ... | ... | ..... | ... | 0·0121 | 2·11 | .... | ... | .... | .... | ..... |
| VI | 0·5348 | ..... | ... | ..... | ... | ... | ... | ..... | ... | 0·0105 | 1·96 | .... | ... | .... | .... | ..... |
| VII | 1·0088 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | 0·0055 | 0·075 | .... | .... | ..... |
| VIII | 0·5490 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | .... | ... | 0·0488 | 1·22 | 1·14 |
| IX | 0·5918 | ..... | ... | ..... | ... | ... | ... | ..... | ... | .... | ... | .... | ... | 0·0517 | 1·20 | 1·12 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·77 | 52·86 | .... | .... | .... | .... | *52·82* |
| H | 7·16 | 7·05 | .... | .... | .... | .... | *7·11* |
| N | .... | .... | 16·78 | 16·83 | .... | .... | *16·80* |
| S | .... | .... | .... | .... | 1·17 | 1·15 | *1·16* |
| O | .... | .... | .... | .... | .... | .... | *22·11* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN H[1], FROM MUSCLE OF SHEEP.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3904 | 0·2441 | 6·95 | 0·7440 | 51·96 | --- | --- | ---- | --- | ---- | --- | ------ | --- | ---- | --- | ---- |
| II | 0·4537 | 0·2854 | 7·01 | 0·8637 | 51·94 | --- | --- | ------ | --- | ---- | --- | ------ | --- | ---- | --- | ---- |
| III | 0·2960 | ------ | --- | ------ | --- | 40·9 | 14·3 | 766·4 | 16·63 | ---- | --- | ------ | --- | ---- | --- | ---- |
| IV | 0·5605 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0096 | 1·71 | ------ | --- | ---- | --- | ---- |
| V | 0·6490 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0108 | 1·67 | ------ | --- | ---- | --- | ---- |
| VI | 1·2095 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | 0·0084 | 0·04 | ---- | --- | ---- |
| VII | 0·5423 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ------ | --- | 0·0464 | 1·18 | 1·14 |
| VIII | 0·5589 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ------ | --- | 0·0553 | 1·35 | 1·31 |
| IX | 0·5233 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ------ | --- | 0·0526 | 1·38 | 1·34 |

*Percentage composition of the ash-free substance.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·86 | 52·82 | ---- | --- | ---- | ---- | *52·84* |
| H | 7·07 | 7·13 | ---- | ---- | ---- | ---- | *7·10* |
| N | ---- | ---- | 16·91 | --- | ---- | ---- | *16·91* |
| S | ---- | ---- | ---- | 1·15 | 1·32 | 1·36 | *1·28* |
| O | ---- | ---- | --- | ---- | ---- | ---- | *21·87* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN H[II], FROM MUSCLE OF SHEEP.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c. c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash. found. gram. | Ash. % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·4075 | 0·2559 | 6·97 | 0·7716 | 51·63 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| II | 0·2374 | 0·1497 | 7·01 | 0·4526 | 51·99 | --- | --- | ------ | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| III | 0·5898 | ------ | --- | ------ | --- | 81·3 | 14·4 | 767·4 | 16·61 | --- | --- | ---- | --- | ---- | --- | ---- |
| IV | 0·2675 | ------ | --- | ------ | --- | 86·0 | 14·9 | 765·5 | 16·56 | ---- | --- | ---- | --- | ---- | --- | ---- |
| V | 0·6164 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0090 | 1·46 | ---- | --- | ---- | --- | ---- |
| VI | 0·5958 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0085 | 1.43 | ---- | --- | ---- | --- | ---- |
| VII | 1·2122 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | 0·0048 | 0·05 | ---- | --- | ---- |
| VIII | 0·5413 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0483 | 1·22 | 1·17 |
| IX | 0·4759 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | --- | 0·0403 | 1·16 | 1·11 |

| | *Percentage composition of the ash-free substance.* | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 52·39 | 52·74 | --- | ---- | ---- | ---- | *52·57* |
| H | 7·08 | 7·11 | ---- | ---- | ---- | ---- | *7·10* |
| N | ---- | ---- | 16·85 | 16·84 | ---- | ---- | *16·84* |
| S | ---- | ---- | ---- | ---- | 1·19 | 1·13 | *1·16* |
| O | ---- | ---- | ---- | ---- | ---- | ---- | *22·33* |
| | | | | | | | *100·00* |

ANALYSIS OF MYOSIN I, FROM MUSCLE OF OX.

| No. | Substance used. gram. | $H_2O$ found. gram. | H % | $CO_2$ found. gram. | C % | N found. c.c. | N found. T. °C. | N found. Pressure. mm. | N % | Ash found. gram. | Ash. % | $BaSO_4$ from the Ash. gram. | S of Ash. % of sub. | $BaSO_4$ after fusion with $KOH + KNO_3$ gram. | S % | S after deducting S of Ash. % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0·3665 | 0·2318 | 7·01 | 0·7053 | 52·48 | --- | --- | ------ | --- | ---- | --- | ---- | ---- | ---- | --- | ---- |
| II | 0·4900 | ------ | --- | 0·9404 | 52·48 | --- | --- | ------ | --- | ---- | --- | --- | ---- | ---- | --- | ---- |
| III | 0·2913 | 0·1868 | 7·13 | 0·5596 | 52·38 | --- | --- | ------ | --- | ---- | --- | ---- | ---- | ---- | --- | --- |
| IV | 0·7746 | ------ | --- | ------ | --- | 109·1 | 15·4 | 759·8 | 16·73 | ---- | --- | ---- | ---- | ---- | --- | ---- |
| V | 0·5013 | ------ | --- | ------ | --- | 70·6 | 14·7 | 757·8 | 16·72 | ---- | --- | ---- | ---- | ---- | --- | ---- |
| VI | 0·7524 | ------ | --- | ------ | --- | 105·9 | 15·0 | 760·7 | 16·77 | ---- | --- | ---- | ---- | ---- | --- | ---- |
| VII | 0·6629 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0043 | 0·65 | ---- | ---- | ---- | --- | ---- |
| VIII | 0·6741 | ------ | --- | ------ | --- | --- | --- | ------ | --- | 0·0046 | 0·68 | ---- | ---- | ---- | --- | ---- |
| IX | 1·0959 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | 0·0020 | 0·025 | ---- | --- | ---- |
| X | 0·4967 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | ---- | 0·0458 | 1·26 | 1·23 |
| XI | 0·5992 | ------ | --- | ------ | --- | --- | --- | ------ | --- | ---- | --- | ---- | ---- | 0·0552 | 1·28 | 1·25 |

*Percentage composition of the ash-free substance.*

| | | | | | | | | | Average. |
|---|---|---|---|---|---|---|---|---|---|
| C | 52·82 | 52·83 | 52·78 | ---- | ---- | ---- | ---- | ---- | *52·79* |
| H | 7·06 | ---- | 7·17 | ---- | ---- | ---- | ---- | ---- | *7·12* |
| N | ---- | ---- | ---- | 16·86 | 16·83 | 16·88 | ---- | ---- | *16·86* |
| S | ---- | ---- | ---- | ---- | ---- | ---- | 1·25 | 1·27 | *1·26* |
| O | | ---- | ---- | ---- | | ---- | | | *21·97* |
| | | | | | | | | | *100·00* |

RÉSUMÉ OF THE ANALYTICAL RESULTS.

| | C | H | N | S | O |
|---|---|---|---|---|---|
| Myosin A, from ox | 52·84 | 7·12 | 16·89 | 1·49 | 21·66 |
| " B, " " | 52·51 | 7·09 | 16·53 | 1·25 | 22·62 |
| " C, " sheep | 53·24 | 7·12 | 16·45 | 1·23 | 21·96 |
| " D', " calf | 52·84 | 7·11 | 17·14 | 1·33 | 21·58 |
| " D'', " " | 52·97 | 7·13 | 16·96 | 1·25 | 21·69 |
| " E, " halibut | 52·39 | 6·97 | 16·74 | ---- | ---- |
| " F', " ox | 52·99 | 7·11 | 16·73 | 1·29 | 21·88 |
| " F'', " " | 52·83 | 7·10 | 16·74 | 1·31 | 22·02 |
| " G', " " | 53·05 | 7·19 | 16·52 | 1·18 | 22·06 |
| " G'', " " | 52·82 | 7·11 | 16·80 | 1·16 | 22·11 |
| " H', " sheep | 52·84 | 7·10 | 16·91 | 1·28 | 21·87 |
| " H'', " " | 52·57 | 7·10 | 16·84 | 1·16 | 22·33 |
| " I, " ox | 52·79 | 7·12 | 16·86 | 1·26 | 21·97 |
| Average | 52·82 | 7·11 | 16·77 | 1·27 | 21·90 |
| Fibrinogen* | 52·93 | 6·90 | 16·66 | 1·25 | 22·26 |
| Fibrin* | 52·68 | 6·83 | 16·91 | 1·10 | 22·48 |
| Paraglobulin* | 52·71 | 7·01 | 15·85 | 1·11 | 23·32 |
| Egg albumin† | 52·18 | 6·93 | 15·81 | 1·87 | 23·21 |

* Hammarsten, Jahresbericht der Thierchemie, vol. x, p. 11.

† Chittenden and Bolton. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 134.

The two products are seen to be practically identical in composition, and show close agreement with the other preparations of myosin.

### *Myosin I, from muscle of ox.*

This, the last product analyzed, was obtained from an ammonium chloride extract of the washed muscle tissue from a freshly killed ox, by dialysis. After being washed and dried at 110° C. it was analyzed with the results shown in the accompanying table.

On comparing now the composition of the thirteen preparations of myosin analyzed, there is seen to be a very close agreement throughout. Further, on comparing the average of the analytical results with Hammarsten's figures for fibrinogen, the composition of the two bodies is seen to be almost identical. Compared with egg albumin, the most striking difference in composition is the low content of sulphur, and even if we assume with Danilewsky* that the sulphur of the ash comes from the sulphur of the myosin the correction therefor would not materially lessen the difference. Myosin also contains nearly one per cent. more nitrogen than egg albumin.

### *Coagulation of myosin solutions.*

According to Kühne,† the coagulation of myosin in a 10 per cent. sodium chloride solution occurs at 55–60° C. Weyl‡ obtained similar results. Danilewsky§ states that a 12–15 per cent. ammonium chloride solution of myosin becomes somewhat turbid at 42–43° C., decidedly turbid at 45–50° C., while at 55° C. a flocculent precipitate separates. The same investigator also found that the more concentrated the ammonium chloride solution was, the lower the temperature at which the turbidity and separation of a precipitate occurred, although never below 40° C. In common with other proteid bodies a slight degree of acidity lowers the temperature of coagulation, while a corresponding degree of alkalinity raises it. According to the recent

* Zeitschrift für Physiologische Chemie, Band v, p. 161.

† Lehrbuch der Physiologischen Chemie, p. 275.

‡ Zeitschrift für Physiologische Chemie, Band i, p. 77.

§ Zeitschrift für Physiologische Chemie, Band v, p. 160.

work of Halliburton,* muscle-clot or myosin is formed from a substance (myosinogen) in the muscle-plasma which coagulates by heat at 47° C. and 56° C., thus indicating that it probably consists of two distinct proteids which coagulate at these temperatures respectively. Further, Halliburton considers that whenever myosin is dissolved in a suitable saline solution, it is at once re-converted into myosinogen, or rather into two proteids which resemble myosinogen in that they have the same heat coagulation temperatures and that they are convertible into myosin by dilution of their saline solutions. In salted muscle-plasma, Halliburton recognizes five distinct proteid bodies, distinguishable by fractional heat coagulation, viz :

47° C., a flocculent, somewhat sticky precipitate.
56° C., a more abundant and very sticky precipitate.
63° C., a finely flocculent precipitate, not sticky.
73° C., a finely flocculent precipitate, not sticky.
A non-coagulable albumose.

Of these, the two proteids coagulating at 47° C. and 56° C., make up the muscle-clot or myosin.

With these preliminary statements, we proceed to the results obtained in our study of the coagulation points of the different myosins, simply prefacing it with the explanation that our experiments were performed previous to reading Halliburton's paper. Naturally, the temperature of coagulation offers the simplest and surest means, in conjunction with the determination of composition, of detecting any difference in the character of the myosins from different sources. And with this as the main object the following experiments were tried. In every experiment, the muscle tissue was chopped quite fine and very thoroughly extracted with water, well thymolized, prior to solution of the myosin with the saline fluid.

We first demonstrated to our satisfaction that a 5 per cent. ammonium chloride solution of myosin invariably coagulates at a lower temperature than a 5 per cent. sodium chloride solution, and further, that the original saline extract of washed muscle shows approximately the same temperature of coagulation as the salt solution of precipitated myosin.

* Journal of Physiology, vol. viii, p. 148.

| | | Turbidity. | Flocculent precipitate. |
|---|---|---|---|
| A. | 5 per cent. $NH_4Cl$ extract of sheep's muscle. | 43° C. | 47° C. |
| B. | 5 per cent. $NH_4Cl$ solution of $H_2O$ precipitate of myosin, from A. | 44° | 48° |
| C. | 5 per cent. NaCl solution of $H_2O$ precipitate of myosin, from A. | 57° | 62° |
| D. | 5 per cent. $NH_4Cl$ extract of ox muscle. | 40° | 44° |
| E. | 5 per cent. NaCl solution of $H_2O$ precipitate of myosin, from D. | 57° | 62° |
| F. | 5 per cent. $NH_4Cl$ extract of lamb's muscle. | 44° | 47° |
| G. | 5 per cent. NaCl solution of $H_2O$ precipitate of myosin, from F. | 51° | 57° |
| H. | 5 per cent. $NH_4Cl$ extract of calf's muscle. | 41° | 44° |
| I. | 5 per cent. NaCl extract of same. | 53° | 56° |
| J. | 5 per cent. $NH_4Cl$ extract of breast muscle from chicken. | 43° | 46° |
| K. | 5 per cent. NaCl extract of same. | 53° | 57° |
| L. | 5 per cent. $NH_4Cl$ extract of leg muscle from chicken. | 45° | 48° |
| M. | 5 per cent. NaCl extract of same. | 56° | 61° |

In all of these trials, the filtrate from the flocculent precipitate failed to show any further coagulation, although the temperature was raised to above 75° C.

The results show a varying difference in the coagulation points of the ammonium and sodium chloride solutions, but on an average the difference amounts to eleven degrees. It further appears that the myosin solutions (in NaCl) from lamb, calf, and chicken muscle have a somewhat lower coagulating point than the corresponding extracts from ox and sheep muscle.

In another series of results, shown in the accompanying table, the same difference in coagulation point shows itself, and it is further seen that the extracts from rabbit's and halibut's muscle have a still lower coagulating point. Further, in this series of experiments, the filtrate from the first coagulum gave a second precipitate on raising the temperature, and in the case of the muscle from ox and lamb, a third coagulum was obtained at 74° C.

COAGULATION OF MYOSIN SOLUTIONS.

| Nature of the Solution. | Coagulation of the Solution. | | Coagulation of the filtrate. | |
|---|---|---|---|---|
| 5 per cent. NaCl. | Turbidity. | Flocculent precipitate. | Turbidity. | Flocculent precipitate. |
| Sheep's muscle. | 57° C. | 63° C. | 68° C. | 73° C. |
| Ox " | 58° | 63° | 66° | 72° |
| Rabbit's " | 52° | 57° | 62° | 66° |
| Pig's " | 56° | 60° | 65° | 70° |
| Lamb's " | 53° | 60° | | |
| Calf's " | 53° | 57° | 66° | 70° |
| Halibut's " | 47° | 53° | 55° | 60° |
| Ox " | 57° | 62° | 64° | 66° |
| Lamb's " | 55° | 62° | 63° | 68° |
| Calf's " | 53° | 59° | 63° | 66° |
| Chicken " (breast) | 53° | 57° | 61° | 67° |
| " " (leg) | 56° | 61° | | |
| Lobster* " | 65° | | | |

The results collectively fail to show the presence of a proteid coagulating at 47° C., given by Halliburton as characteristic of myosin, but they do show a coagulum at 56° C. or thereabouts, corresponding to Halliburton's second proteid. This difference is possibly due to the character of the saline solution, Halliburton having used a magnesium or ammonium sulphate solution. The coagulums obtained at higher temperatures show an approach, at least, to the figures found by Halliburton in his fractional coagulation of salted muscle-plasma.

* Lobster did not yield enough myosin to permit of an exact determination, still a decided turbidity was present at 65° C. The addition of water to the 5 per cent. $NaC_1$ solution, gave a flocculent precipitate which changed to a glairy mass resembling much the white of an egg. On washing with water it became insoluble, but differed in general appearance from similar precipitates from other sources.

A careful study of the preceding results, combined with what has been known concerning the chemical properties of myosin, seems to justify the assumption that myosin, as it occurs throughout the animal kingdom, is a single chemical compound, doubtless formed, as suggested by Halliburton, by the interaction of one or more myosinogens and a ferment body. That myosin is a single body, is supported by the observed agreement in chemical composition and the general uniformity in the temperature of coagulation of myosin from various animal sources, and is furthermore to be inferred from the similarity in function of the tissue in which it occurs most abundantly.

## VIII.—Myosinoses. By W. Kühne and R. H. Chittenden.

Of the primary digestion products of the various proteids, the albumoses from fibrin * and egg albumin † have been more or less carefully examined and analyzed, as also the globuloses,‡ the caseoses,§ and elastinoses. ‖ Further, the vitelloses ¶ have likewise been prepared from crystalline phyto-vitellin and their properties ascertained. With the hope of gradually completing the list we have undertaken a study of the primary digestion products of myosin, the results of which we now present.

For the preparation of myosin, large quantities of finely divided ox muscle were extracted with cold water until the fluid no longer gave reaction for albumin, after which the tissue was placed in an excess of a 15 per cent. ammonium chloride solution, and the myosin ultimately precipitated from the filtered fluid by dialysis.** The extraction of myosin with ammonium chloride, after the method of Danilewsky,†† is far better in every way than the older method of extraction with 10 per cent. sodium chloride, since myosin is dissolved more rapidly and completely by the ammonium salt, even from coarsely divided muscle, and at the same time forms a more easily filterable fluid. Further, the advantage of precipitating the myosin by dialysis in running water, instead of pouring the ammonium chloride solution into a large excess of water, consists in the avoidance of the large volumes of fluid necessary in the preparation of such a large quantity of myosin, while at the same time the myosin is obtained equally free from soluble salts. At the best, however, the preparation of such a quantity of myosin as was needed in the present investigation involved a large amount of labor and a comparatively low temperature, hence compelling us to take the winter season for the work. The united products obtained in this manner were treated ultimately with dilute alcohol, whereby the semi-gelatinous mass was naturally more or less shrunken, and finally with ether.

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* Zeitschrift für Biologie, Band xix, p. 159 and Band xx, p. 11.

† Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 126.

‡ Zeitschrift für Biologie, Band xxii, p. 409.

§ This volume, p. 66. ‖ This volume, p. 19.

¶ Ueber vitellosen, by Dr. R. Neumeister. Zeitschrift für Biologie. Band xxiii, p. 2.

** See the preceding article.

†† Zeitschrift für Physiologische Chemie, Band v, p. 158.

A sample of the product, carefully dried at 110° C. until of constant weight, yielded the following results on analysis.

### *Myosin.*

I. 0·3665 gram substance gave 0·2313 gram $H_2O$ = 7·01 per cent. H and 0·7053 gram $CO_2$ = 52·48 per cent. C.

II. 0·4900 gram substance gave 0·9404 gram $CO_2$ = 52·48 per cent. C.

III. 0·2913 gram substance gave 0·1868 gram $H_2O$ = 7·13 per cent. H and 0·5596 gram $CO_2$ = 52·38 per cent. C.

IV. 0·7746 gram substance gave 109·1 c.c. N at 15·4° C. and 759·8 mm pressure = 16·73 per cent. N.

V. 0·5013 gram substance gave 70·6 c.c. N at 14·7° C. and 757·8 mm pressure = 16·72 per cent. N.

VI. 0·7524 gram substance gave 105·9 c.c. N. at 15·0° C. and 760·7 mm pressure = 16·77 per cent. N.

VII. 0·6629 gram substance gave 0·0043 gram ash = 0·65 per cent.

VIII. 0·6741 gram substance gave 0·0046 gram ash = 0·68 per cent.

IX. The ash from 1·3370 grams substance gave 0·002 gram $BaSO_4$ = 0·02 per cent. S.

X. 0·4967 gram substance gave by fusion with KOH + $KNO_3$, after Hammarsten's method, 0·0458 gram $BaSO_4$ = 1·26 per cent. S; deducting 0·02 per cent. S of ash = 1·24 per cent.

XI. 0·5992 gram substance gave after fusion with KOH + $KNO_3$, 0·0552 gram $BaSO_4$ = 1·28 per cent. S; deducting 0·02 per cent. = 1·26 per cent.

*Percentage composition of ash-free myosin.*

| | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|
| C | 52·82 | 52·83 | 52·73 | ---- | ---- | ---- | ---- | ---- | 52·79 |
| H | 7·06 | ---- | 7·17 | ---- | ---- | ---- | ---- | ---- | 7·12 |
| N | ---- | ---- | ---- | 16·86 | 16·83 | 16·88 | ---- | ---- | 16·86 |
| S | ---- | ---- | ---- | ---- | ---- | ---- | 1·25 | 1·27 | 1·26 |
| O | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 21·97 |
| | | | | | | | | | 100·00 |

The ash consisted almost entirely of calcium phosphate.

### *Digestion of Myosin.*

The myosin purified as described with alcohol and ether, proved so resistant to the action of pepsin-hydrochloric acid, that its diges-

tion could be accomplished only by repeated treatment with the ferment. The gastric juice at first emyloyed was prepared by warming 120 grams of mucous membrane from a pig's stomach with 1200 c. c. 0·4 per cent. hydrochloric acid for 24 hours at 41° C., filtration through paper, dilution of the acid fluid with an equal volume of water and further exposure to a temperature of 40° C. for four days, in order to convert any dissolved albumoses into peptone.

The digestive fluid so obtained contained 0·2 per cent. HCl and 0·5 per cent. solid matter.

200 grams of finely powdered myosin were placed in 2 litres of this gastric juice, 2 litres of 0·2 per cent. hydrochloric acid added and the whole warmed at 40° C. for two days. As only a little of the myosin appeared to be dissolved, the acidity was increased to 0·4 per cent. and the mixture continued at 40° C. for 24 hours longer. Although a large residue still remained undissolved, the entire mixture was made neutral with sodium hydroxide and strained through a cloth filter. The undigested residue, together with the abundant neutralization precipitate, was again warmed for several days at 40° C. with 2 litres of 0·4 per cent. hydrochloric acid containing 7·5 grams of scrapings from a stomach mucous membrane. After stopping the action of the pepsin by neutralization, this second digestive fluid was added to the first. In spite of the energetic action of the pepsin (tested by allowing a little of the solution to act on boiled fibrin), there still remained considerable undissolved substance together with considerable neutralization precipitate, the whole apparently very resistant to the action of the ferment. In the united solutions there was present less than 100 grams of organic matter, of which it is fair to presume about 10·5 grams consisted of impurity in the form of substances from the stomach membrane. When it is remembered, however, that well prepared gastric juice contains only a very small amount of substances precipitable by the salts used in separation of the proteoses, it is fair to assume that this impurity in the digestive fluid is unimportant in the study of the myosinoses. In all, nearly 60 grams of myosinoses were obtained.

For separation of the myosinoses, the united neutral filtrates were concentrated to about one-sixth of their volume and saturated with crystals of rock salt, by which the fluid was converted into a gelatinous mass. On adding saturated salt solution to a portion of the filtered fluid it was rendered decidedly turbid; consequently, three volumes of a saturated sodium chloride solution were added to the mixture, after which it was found that neither salt in substance or in solution

would give any further turbidity. From this it is evident that the neutral digestive fluid is not completely precipitated by simple saturation with sodium chloride, but that complete precipitation is reached only when the absolute quantity of salt stands in a certain proportion to the proteoses present.

After this separation of the first portion of the myosinoses, which would naturally consist of proto, hetero, and dysmyosinose, the remainder were precipitated first with salt-saturated 30 per cent. acetic acid and then, after removal of the sodium chloride by dialysis, with neutral ammonium sulphate. In the solution remaining from this last precipitation, peptone was detected by the biuret reaction.

### *Protomyosinose.*

The sodium chloride precipitate, after thorough washing with saturated salt solution, dissolved almost entirely on being rubbed up with water.

What did remain undissolved, showed the reactions of dysalbumose. It was insoluble in water and in salt solutions of all strengths, but easily soluble in 0·1 per cent. hydrochloric acid, much more difficultly soluble in 0·5 per cent. sodium carbonate, by neutralization only partially precipitated and gave the biuret reaction. From the filtrate of the neutralized hydrochloric acid solution, some heteromyosinose in the form of a flocculent precipitate was obtained by dialysis. This hetero body was soluble in dilute sodium chloride solutions, insoluble in water, precipitable by salt in substance, and after the manner of the albumoses gave a precipitate with nitric acid in the cold, which disappeared as the mixture was warmed, reappearing as the solution cooled. This heteromyosinose, formed from dysmyosinose by a process of retrogression, amounted to considerable; about 1·5 grams.

### *Reactions of Protomyosinose.*

In order to purify protomyosinose the aqueous solution of the substance was freed from sodium chloride by dialysis, by which only traces of heteromyosinose separated, and the solution evaporated to a thin syrup. The fluid was filtered from a slight flocculent albumin-like precipitate insoluble in hot water, somewhat further concentrated and the pure myosinose separated by alcohol. After washing with alcohol and ether it appeared as a light, white powder. The yield amounted to about ten grams.

The reactions of protomyosinose agree in general with those of protoalbumose, but with one important difference, viz: that an aqueous solution of the former, free from salt, of whatever concentration, is not rendered turbid by nitric acid. Addition of even a little sodium chloride to the acid fluid, however, is sufficient to cause a heavy precipitate, soluble as the mixture is warmed but reappearing as the solution cools. The precipitate is also soluble in an excess of the acid in the cold.

Protomyosinose is readily soluble in distilled water, the solution showing a weak, but unquestionably alkaline reaction. In this solution, as well as in a solution rendered acid by acetic acid, cupric sulphate produces a heavy turbidity, which on boiling almost entirely disappears. Acetic acid and potassium ferrocyanide produce a heavy precipitate, insoluble in glacial acetic acid. Neutral lead acetate gives no precipitate. Basic lead acetate and mercuric chloride both produce a heavy turbidity. An aqueous solution of the myosinose boiled with sodium hydroxide and lead acetate is colored deep brown or black; with sodium hydroxide and cupric sulphate a beautiful red. Concentrated sodium hydroxide, as in protoelastose, produces a heavy flocculent, gelatinous precipitate. By saturation of an aqueous solution of protomyosinose with sodium chloride, only a portion of the substance is precipitated; the portion remaining dissolved is precipitable by acid.

The composition of the substance, dried at 110° C. until of constant weight, is shown by the following analysis.

*Protomyosinose.*

I. 0·3722 gram substance gave 0·2387 gram $H_2O$ = 7·12 per cent. H and 0·7090 gram $CO_2$ = 51·95 per cent. C.

II. 0·4239 gram substance gave 0·2688 gram $H_2O$ = 7·05 per cent. H and 0·8048 gram $CO_2$ = 51·77 per cent. C.

III. 0·3319 gram substance gave 0·2138 gram $H_2O$ = 7·15 per cent. H and 0·6308 gram $CO_2$ = 51·83 per cent. C.

IV. 0·4798 gram substance gave 67·5 c. c. N at 14·8° C. and 762·1 mm pressure = 16·79 per cent. N.

V. 0·5663 gram substance gave 78·3 c. c. N at 15·1° C. and 768·2 mm pressure = 16·64 per cent. N.

VI. 0·4429 gram substance gave 62·7 c. c. N at 15·8° C. and 758·2 mm pressure = 16·77 per cent. N.

VII. 0·5494 gram substance gave 0·0062 gram ash = 1·13 per cent.

VIII. 0·7080 gram substance gave 0·0081 gram ash = 1·14 per cent.

IX. The ash from 1·2574 grams substance gave 0·0132 gram $BaSO_4$ = 0·14 per cent. S.

X. 0·4183 gram substance gave after fusion with KOH + $KNO_3$ 0·0450 gram $BaSO_4$ = 1·47 per cent. S; after deducting 0·14 per cent. S of the ash = 1·33 per cent.

XI. 0·3963 gram substance gave after fusion with KOH + $KNO_3$ 0·0411 gram $BaSO_4$ = 1·42 per cent. S; deducting 0·14 per cent. = 1·28 per cent.

*Percentage composition of ash-free protomyosinose.*

| | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|
| C | 52·53 | 52·35 | 52·41 | .... | .... | .... | .. | .... | 52·43 |
| H | 7·19 | 7·12 | 7·22 | .... | .... | .... | .... | .... | 7·17 |
| N | .... | .... | .... | 16·98 | 16·83 | 16·96 | .... | .... | 16·92 |
| S | .... | .... | .... | .... | .... | .... | 1·35 | 1·30 | 1·32 |
| O | .... | .... | .... | .... | .... | .... | .... | .... | 22·16 |
| | | | | | | | | | 100·00 |

The ash contained only calcium phosphate and sulphate, with a little ferric oxide.

## *Deuteromyosinose.*

This myosinose, as we have before mentioned, was obtained mainly by saturation with ammonium sulphate. After removal of the greater portion of protomyosinose by saturation with salt as described, deuteromyosinose was in part precipitated by salt-saturated acetic acid, but this precipitate could not be used on account of the large amount of proto body precipitated with it, as shown by the cupric sulphate reaction. This method of treatment, however, although necessitating the loss of considerable deuteromyosinose, enabled us to remove the proto body completely and thus ensure a pure specimen of deuteromyosinose on treatment of the filtrate with ammonium sulphate. Naturally, before saturating the fluid with the ammonium salt, the sodium chloride was removed by dialysis. The myosinose thus precipitated by saturation with ammonium sulphate was dissolved in water and dialyzed until the sulphate was entirely removed, or to such an extent that the fluid gave only the slightest turbidity with barium chloride even on long standing. We hastened the removal of the sulphate by repeated evaporation and renewed dialysis of the concentrated fluid. The substance was finally precipitated from the suitably concentrated fluid, as a white powder, by alcohol and washed with alcohol and ether. It weighed forty-five grams.

*Reactions of deuteromyosinose.*

Like the preceding myosinose, this body also reacts alkaline in an aqueous solution and this property exercises more or less of an influence on certain of its reactions. In order to convince ourselves of the absence of traces of either proto or heteromyosinose, we paid particular attention to the behavior of the deutero body towards the cupric sulphate reaction, which according to Neumeister's observations is a decisive test on this point. A fairly concentrated solution of our preparation was not rendered turbid by cupric sulphate in the cold, but after boiling and then cooling the mixture, a slight turbidity appeared. Solutions of the substance so concentrated as to be almost syrupy, gave a slight turbidity at once, the turbidity disappearing when the solution was heated and reappearing as the fluid cooled. By partial neutralization of the alkalinity of the myosinose solution, leaving the fluid, however, still alkaline to delicate test papers, precipitation by cupric sulphate was entirely prevented. As protomyosinose is precipitated by the copper salt equally well in an acid fluid, we are led to consider our deuteromyosinose entirely free from this impurity.

In general, deuteromyosinose shows much the same reactions as deuteroalbumose, but is somewhat different from the latter in that it is more difficultly precipitable. Acetic acid and nitric acid produce a precipitate only after addition of sodium chloride to saturation. Acetic acid and potassium ferrocyanide give a decided turbidity, not soluble in glacial aectic acid. Basic lead acetate and mercuric chloride both produce a precipitate, insoluble in excess of the reagent. Cold nitric acid quickly produces an intense yellow color. The biuret reaction comes out distinctly, but on boiling the myosinose with sodium hydroxide and lead acetate only a faint browning of the fluid is obtained.

The composition of the substance, dried at 110° C., is shown by the following analysis:

*Deuteromyosinose.*

I. 0·2121 gram substance gave 0·1394 gram $H_2O$ = 7·30 per cent. H and 0·3896 gram $CO_2$ = 50·09 per cent. C.

II. 0·2115 gram substance gave 0·1390 gram $H_2O$ = 7·30 per cent. H and 0·3891 gram $CO_2$ = 50·12 per cent. C.

III. 0·3830 gram substance gave 53·6 c. c. N at 15·0° C. and 762·5 mm pressure = 16·71 per cent. N.

IV. 0·2940 gram substance gave 41·4 c. c. N at 15·7° C. and 759·7 mm pressure = 16·72 per cent. N.

V. 0·4572 gram substance gave 0·0080 gram ash = 1·77 per cent.

VI. 0·5462 gram substance gave 0·0094 gram ash = 1·72 per cent.

VII. The ash from 1·0034 grams substance gave 0·0116 gram $BaSO_4$ = 0·15 per cent. S.

VIII. 0·3343 gram substance gave after fusion with KOH + $KNO_3$ 0·0315 gram $BaSO_4$ = 1·29 per cent. S; after deducting 0·15 per cent. S of the ash = 1·14 per cent.

IX. 0·4050 gram substance gave after fusion with KOH + $KNO_3$ 0·0420 gram $BaSO_4$ = 1·42 per cent. S; deducting 0·15 per cent. = 1·27 per cent.

*Percentage composition of ash-free deuteromyosinose.*

| | | | | | | | Average. |
|---|---|---|---|---|---|---|---|
| C | 50·95 | 50·98 | ---- | ---- | ---- | ---- | 50·97 |
| H | 7·42 | 7·42 | ---- | ---- | ---- | ---- | 7·42 |
| N | ---- | ---- | 17·00 | 17·01 | ---- | ---- | 17·00 |
| S | ---- | ---- | ---- | ---- | 1·16 | 1·28 | 1·22 |
| O | ---- | ---- | ---- | ---- | ---- | ---- | 23·39 |
| | | | | | | | 100·00 |

The ash consisted only of calcium phosphate and sulphate, with some oxide of iron.

This is as far as we have been able at present to carry our study of the myosinoses, since hetero and dysmyosinose appear to have been present in the digestions only in very small quantity. Myosin purified by alcohol, as was the preparation employed by us, is so difficultly digestible that it is attacked only by the most energetic pepsin mixture, and this has the disadvantage of rapidly converting hetero and protoproteose, which according to Neumeister's * investigations are formed in the beginning of digestion, into the deutero body; consequently in the present instance we could expect a large amount only of deuteromyosinose. Corresponding with this view, we obtained for 45 grams of deuteromyosinose, only 10 grams of protomyosinose and but 3 grams of hetero and dysmyosinose. Further, the unavoidable loss attending the separation of these bodies was probably greater with deuteromyosinose than with the others. Portions of the fluid from the first and second digestion, tested before they were united, showed also a difference in that the fluid from the first digestion con-

* Loc. cit.

tained far more proto and dysmyosinose than the latter, which, on the other hand, was particularly rich in deuteromyosinose. From this it is evident how an albuminous body can in one sense be difficultly digestible, in that its solution takes place slowly and its primary cleavage products form gradually, and yet the latter be further transformed, under the continued action of the ferment, far more readily and completely. Our conception of digestibility needs therefore to be broadened, after having for so long embraced simply the time required for solution of the proteid, or, in the case of pepsin digestion, the hardly attainable extreme of complete conversion into peptone.

On comparing now the results of the analyses of myosin and the two myosinoses in the following table,

| | C | H | N | S | O |
|---|---|---|---|---|---|
| Myosin | 52·79 | 7·12 | 16·86 | 1·26 | 21·97 |
| Protomyosinose | 52·43 | 7·17 | 16·92 | 1·32 | 22·16 |
| Deuteromyosinose | 50·79 | 7·42 | 17·00 | 1·22 | 23·39 |

there is seen to be only a small difference in composition between myosin and protomyosinose. The content of carbon in the latter is only 0·36 per cent. less than in the former, that of nitrogen 0·06 per cent. greater and oxygen only 0·17 per cent. greater. Between deuteromyosinose and the undigested proteid, on the other hand, there is a far greater difference in composition, the content of carbon being 1·82 per cent. less, while nitrogen is 0·14 per cent. greater and oxygen 1·42 per cent. greater. In hydrogen, both myosinoses show a small increase over myosin, while the content of sulphur is practically unaltered.

That portion of the myosin, which was apparently not further alterable by gastric juice, together with the somewhat large neutralization precipitate, we attempted to digest by the action of trypsin. This was only partially successful, for although the trypsin solution consisted of an extract from 20 grams of dry pancreas in 2 litres of 0·4 per cent. sodium carbonate, and the proteid matter was warmed with it at 40° C. for six days, we were not able to bring more than half of the material into solution. The digestive fluid behaved somewhat peculiarly, in that with a certain excess of acetic acid it gave a fine pulverulent white precipitate. After removal of this substance, the solution gave no turbidity whatever with sodium chloride, or with sodium chloride and nitric acid, and only a very slight one with ammonium sulphate ; hence it contained no myosinoses. Peptone, however, was formed in considerable quantity.

## IX.—The Relative Absorption of Nickel and Cobalt. By R. H. Chittenden and Charles Norris, Jr., Ph.B.

When nickel and cobalt were first discovered they were supposed to be possessed of decided toxic properties, and nickel particularly was looked on as more poisonous than copper. Examination, however, of many of the supposed cases of nickel poisoning led to the view that toxic action was due, mainly at least, to the presence of arsenical impurities, with which German nickel particularly was known to be contaminated. Gradually, therefore, the view has become widespread that nickel and cobalt are no more poisonous than iron, with which chemically they are so closely related. We have not, however, been able to find many very definite statements regarding their physiological or toxic action. Blake * in his study of the relation between isomorphism, molecular weight, and physiological action, places the sulphates of nickel and cobalt in the same group with copper, zinc, iron, etc., and further arranges cobalt, copper and zinc together in a sub-group, on account of their arresting the action of the heart and preventing the coagulation of the blood. Nickel, however, is placed with manganese on account of its exerting a marked influence on the nervous system. As to the intensity of their physiological action, Blake apparently considers nickel and copper of the same strength, while cobalt is figured as one-twentieth stronger. Both salts, however, kill by arresting the action of the heart and in lethal action cobalt stands first.† Woodman and Tidy ‡ state that 30 grains of the oxide of cobalt given to a dog proved fatal in a few hours, whilst 3 grains of the sulphate injected into a vein proved fatal in four days. With nickel, the same writers state that vomiting is freely induced in a dog by a dose of 20 grains of the sulphate, whilst 10 grains injected into the jugular vein will destroy life instantly. Finally, Brunton and Cash have found that nickel and cobalt, like most other metallic salts, cause slight contraction of the blood vessels.§

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* American Journal of Science and Arts, vol. vii, p. 194.

† See Brunton's Pharmacology and Therapeutics, p. 51.

‡ Forensic Medicine and Toxicology, p. 171 and p. 214.

§ Brunton's Pharmacology, p. 246.

Our experiments have been conducted wholly upon rabbits, the main object being to study the distribution of the absorbed poison. The salts used were chemically pure cobalt and nickel nitrates, dried over sulphuric acid. They were administered by mouth in gelatin capsules.

We first endeavored to gain some idea of the relative toxic action of the two salts. For this purpose two rabbits, weighing 2 kilos. each, were dosed as follows :

*Experiment I.*

| | | Rabbit A. | | | Rabbit B. | | |
|---|---|---|---|---|---|---|---|
| Oct. 1, | 10 a.m. | 0·150 | gram | $Ni(NO_3)_2$ | 0·150 | gram | $CO(NO_3)_2$ |
| " 1, | 4 p.m. | 0·102 | " | " | 0·100 | " | " |
| " 2, | 10 a.m. | 0·150 | " | " | 0·150 | " | " |
| " 2, | 5 p.m. | 0·101 | " | " | 0·100 | " | " |
| " 3, | 9 a.m. | 0·200 | " | " | 0·200 | " | " |
| " 3, | 4 p.m. | 0·200 | " | " | 0·200 | " | " |
| " 4, | 9 a.m. | 0·251 | " | " | 0·250 | " | " |
| " 4, | 5 p.m. | 0·250 | " | " | 0·250 | " | " |
| " 5, | 10 a.m. | 0·350 | " | " | 0·350 | " | " |
| " 5, | 4 p.m. | 0·350 | " | " | 0·350 | " | " |
| " 6, | 9 a.m. | 0·500 | " | " | 0·500 | " | " |
| " 6, | 3 p.m. | 0·500 | " | " | 0·500 | " | " |
| | | 3·104 grams. | | | 3·100 grams. | | |

Both rabbits died on the morning of the 7th, apparently from heart failure. The urine was examined each day, but in both cases was entirely free from either sugar or albumin. On the 3d, the cobalt rabbit appeared troubled with involuntary micturition and defecation, and on the 5th there was quite pronounced partial paralysis of the hind quarters. The appetite remained good up to the 6th. On the 6th instant, after administration of 500 milligrams of the cobalt salt, there was a loose diarrhœa continuous till death, decided paralysis of the hind legs, total loss of appetite with a decided lowering of the body temperature. After death, the body weight was found to have diminished three-fifths of a kilo. With the nickel rabbit, there was no diarrhœa whatever and the paralysis of the hind legs, visible on the 6th, was not as pronounced as with cobalt. The loss of body weight was the same as in the cobalt rabbit.

On post-mortem, the only noticeable abnormal feature with either cobalt or nickel was a slight congestion of the lining membrane of the stomach and intestines. The stomach, however, was found full of undigested food, as if the salt had interfered with the digestive process.

It is thus seen that neither the cobalt or nickel salt can be called a violent poison, since comparatively large amounts are required to produce a toxic effect, and even then the action is somewhat slow. This is still more clearly seen in the next experiment.

*Experiment II*, with cobalt.

A vigorous black doe, weighing 2 kilos., was dosed as follows:

| | | | | | |
|---|---|---|---|---|---|
| Oct. | 8, | 0·200 | gram | cobalt | nitrate. |
| " | 9, | 0·400 | " | " | " |
| " | 10, | 0·600 | " | " | " |
| " | 11, | 0·700 | " | " | " |
| " | 12, | 0·250 | " | " | " |
| " | 13, | 0·250 | " | " | " |
| " | 14, | 0·250 | " | " | " |
| " | 15, | 0·550 | " | " | " |
| " | 16, | 1·000 | " | " | " |
| " | 17, | 1·500 | " | " | " |
| " | 18, | 2·000 | " | " | " |
| | | 7·700 grams. | | | |

The animal died on the 19th, of heart failure. On the 11th, when 7 decigrams of the salt were given, the animal appeared sickly, with loss of appetite, high rectal temperature, etc., but by diminishing the dose of cobalt the animal rapidly recovered. On the 17th, however, with increase in the dose of salt there was diarrhœa, with a slight indication of paralysis of the extremities. At no time did the urine contain either sugar or albumin. There was a decided loss of body weight, nearly one-third. On post-mortem, the stomach and small intestines were found somewhat inflamed, and the liver showed signs of a slight fatty degeneration.

Immediately on the death of the animal the internal organs were removed and the absorbed cobalt determined.

The method of analysis, both for cobalt and nickel, was as follows: The finely divided tissue was oxidized with dilute hydrochloric acid and potassium chlorate, after the usual method. From the solution so obtained, chlorine was removed by evaporation, the fluid made alkaline with ammonia and the cobalt or nickel precipitated by a stream of hydrogen sulphide gas. The washed sulphide, after ignition, was then dissolved in nitro-hydrochloric acid, the free acid entirely removed by heat and the chloride converted into sulphate by addition of concentrated sulphuric acid. Ultimately, the sulphuric acid solution of sulphate was diluted somewhat with water, made strongly alkaline with ammonia and the metal separated by electrolysis.

Following are the amounts of absorbed cobalt found in experiment II:

| | Total weight of organ. grams. | Weight of Co. milligrams. | Co per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Stomach and contents | 81·75 | 57·5 | 70·34 |
| Small intestines | 43·90 | 13·8 | 31·44 |
| Large intestines | 26·40 | 40·2 | 152·10 |
| Cæcum | 154·00 | 302·5 | 196·43 |
| Liver | 45·75 | 8·9 | 19·43 |
| Kidneys | 10·30 | lost | ---- |
| Heart | 8·80 | 0·7 | 7·95 |
| Lungs | 5·82 | 0·9 | 15·46 |
| Muscle of legs | 100·00 | 1·9 | 1·90 |
| Muscle of back | 27·45 | 1·5 | 5·46 |
| Brain | 8·75 | 0·8 | 6·45 |
| Spinal cord | 3·63 | | |

Considering the large amount of cobalt nitrate administered, the extreme solubility of the salt, and the length of time intervening between the first and last dose, it is somewhat surprising that the amount absorbed was not greater. Evidently a large portion of the cobalt passes directly through the alimentary canal, probably combining with the proteid matter of the food to form an insoluble and indigestible compound.

### *Experiment III*, with nickel.

A black and white doe of 4 kilos. body weight was dosed as follows:

| | | | | |
|---|---|---|---|---|
| Oct. 15, | 0·200 | gram | nickel | nitrate. |
| " 16, | 0·400 | " | " | " |
| " 17, | 0·600 | " | " | " |
| " 18, | 0·800 | " | " | " |
| " 19, | 0·500 | " | " | " |
| | 2·500 grams. | | | |

On the morning of the 20th, the animal was found dead, the only noticeable symptoms having been general weakness, loss of appetite and diarrhœa. The loss of body weight was quite pronounced, amounting in the six days to 1·4 kilos. The urine was entirely free from albumin and sugar. There was a little inflammation of the stomach. In this experiment, the toxic action would appear to have been greater than that of the cobalt in the preceding experiment. The distribution of the absorbed poison was as follows:

| | Total weight of organ. grams. | Weight of Ni. milligrams. | Ni per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Stomach and contents | 115·00 | 12·2 | 10·68 |
| Small intestines | 67·87 | 11·4 | 16·88 |
| Large intestines | 37·45 | 3·9 | 10·41 |
| Cæcum | 190·00 | 9·4 | 4·95 |
| Liver | 87·50 | 5·1 | 5·83 |
| Kidneys | 18·85 | 0·8 | 4·24 |
| Heart | 8·90 | 1·3 | 14·60 |
| Lungs | 14·15 | 0·9 | 6·36 |
| Muscle of legs | 292·00 | 1·2 | 0·41 |
| Muscle of back | 129·00 | 3·8 | 2·95 |
| Brain | 8·75 | 2·1 | 24·00 |
| Spinal cord | 5·00 | 1·0 | 20·00 |
| Spleen | 2·00 | 0·6 | 30·00 |

The amount of nickel found in the alimentary tract is naturally not so large as in the case of cobalt, where the final doses were larger and the diarrhœa not so bad. Of the absorbed nickel, the distribution is essentially the same as with cobalt. The amount in the kidneys and liver is not as large as would be expected from the size of the doses and the soluble character of the salt. It suggests that only a small portion of the salt given is absorbed, and that elimination goes on with comparative slowness. Quite striking is the peculiar distribution of the nickel in the muscle tissue, the amount in the muscles from the back being seven times as large as in the leg muscles. The same peculiarity is likewise noticeable with cobalt. Also noticeable is the comparatively large amount in the brain and spinal cord, more of the poison in proportion to the weight of the organ being found here than in either the liver or kidneys.

*Experiment IV*, with cobalt and nickel.

In this experiment, two rabbits of approximately the same body weight were dosed with nickel and cobalt respectively as follows :

| | *Rabbit A.* | *Rabbit B.* |
|---|---|---|
| Nov. 1, | 0·200 gram $CO(NO_3)_2$. | 0·200 gram $Ni(NO_3)_2$. |
| " 2, | 0·150 " " | 0·350 " " |
| " 3, | 0·250 " " | 0·200 " " |
| " 4, | 0·400 " " | 0·300 " " |
| " 5, | 0·250 " " | 0·500 " " |
| " 6, | 0·800 " " | 0·300 " " |
| " 7, | 0·500 " " | 0·800 " " |
| " 8, | 0 " " | 0·500 " " |
| | 2·550 grams. | 3·150 grams. |

Both animals were found dead on the 9th. In the cobalt rabbit, the stomach, lungs, kidneys and brain were found more or less congested and there was considerable diarrhœa before death. In the nickel rabbit, there was no diarrhœa and but little congestion. Rectum was found full of hard fæces.

Following is the distribution of the poison in the two rabbits.

*Rabbit A, cobalt.*

| | Total weight of organ. grams. | Weight of Co. milligrams. | Co per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Stomach and contents | 76·65 | 10·8 | 14·09 |
| Small intestines | 58·70 | 7·5 | 12·77 |
| Large intestines | 20·10 | 10·9 | 54·23 |
| Cæcum | 108·00 | 50·5 | 46·76 |
| Liver | 91·08 | 3·3 | 3·62 |
| Kidneys | 13·55 | 0·8 | 5·90 |
| Heart | 13·37 | 0·9 | 6·73 |
| Lungs | 9·62 | 1·3 | 13·50 |
| Muscle of legs | 196·00 | 1·2 | 0·61 |
| Muscle of back | 95·85 | 1·7 | 1·77 |
| Brain | 8·87 | 1·2 | 13·53 |
| Spinal cord | 4·95 | 0·9 | 18·18 |

*Rabbit B, nickel.*

| | Total weight of organ. grams. | Weight of Ni. milligrams. | Ni per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Stomach and contents | 64·80 | 18·4 | 28·39 |
| Small intestines | 52·30 | 2·0 | 3·82 |
| Large intestines | 12·10 | 2·0 | 16·53 |
| Liver | 70·00 | 5·6 | 8·00 |
| Kidneys | 11·20 | 0·8 | 7·05 |
| Heart | 6·72 | 0·8 | 11·90 |
| Lungs | 7·92 | 2·1 | 26·51 |
| Muscle of leg | 157·00 | 1·1 | 0·70 |
| Muscle of back | 82·00 | 2·0 | 2·44 |
| Brain | 9·00 | 1·2 | 13·33 |
| Spinal cord | 3·98 | 1·7 | 42·71 |

Very noticeable in both of these results, as in the preceding experiments, is the comparatively large amount of poison in the brain and spinal cord, also the same relative distribution in the muscle tissue of the legs and back previously commented upon. The large amount of poison in the lungs and heart, as contrasted with the liver and kidneys, is also quite noticeable.

### *Experiment V*, with nickel.

In this experiment, 1·8 grams of nickel nitrate were given by mouth during five days to a rabbit of 1·5 kilos. weight, the individual doses being of about the same size as in preceding experiments. On the sixth day, the animal died of heart failure. Following is the distribution of the poison.

| | Total weight of organ. grams. | Weight of Ni. milligrams. | Ni per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Stomach and contents | 73·00 | 3·2 | 4·38 |
| Small intestines | 59·10 | 1·8 | 3·04 |
| Large intestines | 36·40 | 9·9 | 27·20 |
| Cæcum | 176·00 | 36·5 + | 20·74 + |
| Liver | 58·00 | 8·1 | 13·96 |
| Kidneys | 10·27 | 0·8 | 7·79 |
| Heart | 7·42 | 1·3 | 16·63 |
| Lungs | 6·52 | 0·2 | 3·06 |
| Muscle of legs | 97·30 | 2·6 | 2·67 |
| Muscle of back | 35·00 | 3·7 | 10·57 |

### *Experiment VI*, with cobalt.

In this experiment, an attempt was made to ascertain something regarding the elimination of the cobalt through the kidneys. For this purpose, the urine was collected each day and the cobalt determined by the same method as used in the analysis of the organs. The rabbit (body weight 2·5 kilos.) was dosed as follows :

| | | *Examination of the 24 hours' urine.* |
|---|---|---|
| Nov. 27, | 0·100 gram $CO(NO_3)_2$. | 30 c.c. contained 1·3 milligrams Co. |
| " 28, | 0·200 " " | 50 " " 6·1 " " |
| " 29, | 0 " " | 73 " " 6·2 " " |
| " 30, | 0·300 " " | |
| Dec. 1, | 0·200 " " | |
| " 2, | 0 " " | 120 " " 23·3 " " |
| " 3, | 0·400 " " | |
| " 4, | 0·400 " " | |
| " 5, | 0·400 " " | |
| " 6, | 0·400 " " | |
| " 7, | 0·400 " " | |
| " 8, | 0·500 " " | |
| " 9, | 0 " " | |
| " 10, | 0·500 " " | |
| " 11, | 0·500 " " | |
| | 4·300 grams. | |

The animal died on the 12th. On the last day, 15 c. c. of thick, dark brown, viscid urine were voided which contained 1·8 milligrams of cobalt. 7·15 grams of fæces excreted during the last 24 hours were found to contain about 100 milligrams of cobalt. Obviously a large amount of the cobalt passes directly through the alimentary canal, while of the absorbed portion considerable is eliminated through the urine, much more indeed than the small amounts found in the kidneys would appear to warrant.

On analysis of the organs of this rabbit, the following results were obtained:

| | Total weight of organ. grams. | Weight of Co. milligrams. | Co per 100 grams of tissue. milligrams. |
|---|---|---|---|
| Liver | 66·00 | 2·6 | 3·94 |
| Spleen | 0·40 | 0·5 | 125·00 |
| Kidneys | 15·30 | 0·5 | 3·27 |
| Heart | 10·73 | 0·1 | 0·93 |
| Lungs | 7·02 | 1·0 | 14·24 |
| Muscle of leg | 140·00 | 9·8 | 7·00 |
| Muscle of back | 130·00 | 4·7 | 3·61 |
| Brain | 8·35 | 1·5 | 17·96 |
| Spinal cord | 4·10 | 2·0 | 48·78 |

Here, as in many of the preceding cases, the lungs contain a higher percentage of the poison than either the liver or kidneys, while the brain and spinal cord contain a still higher percentage.

With this rabbit, convulsions were noticed shortly before death and the breathing was very labored, as if the respiratory muscles were affected.

An experiment, similar to the preceding, tried with nickel, led to a like result as regards the elimination of the poison. The first 24 hours' urine (25 c. c.) contained 1·6 milligrams of nickel, while the portion (70 c. c.) passed during the 24 hours preceding death contained 8·1 milligrams of the metal. Further, 3·5 grams of fæces excreted during the last 24 hours contained 17·5 milligrams of nickel.

It is obvious from the foregoing that soluble nickel and cobalt salts are possessed of decided toxic properties, but that their poisonous action is somewhat slow and manifested only when comparatively large amounts of the salts are administered. Further, so far as our experiments show, the two salts act very much alike. Both apparently cause death by stopping the action of the heart and also produce more or less disturbance in the alimentary tract, interfering with digestion, producing more or less inflammation of the mucous

membrane of the stomach and intestines, and causing a more or less persistent diarrhœa. Unlike uranium, these salts have no apparent action on the kidneys or liver, sections of hardened tissue from these organs showing no change of structure. Further, in every case the urine of the poisoned animal was entirely free from sugar and albumin throughout the experiment. Both salts tend to produce a partial paralysis of the extremities, more pronounced possibly with cobalt than with nickel. They enter the circulation quickly, are rapidly distributed to all parts of the body and are in turn more or less rapidly eliminated by the kidneys. Considerable, however, evidently passes directly through the alimentary tract and is excreted through the fæces.

Both salts appear to raise the internal body temperature quite decidedly, the rectal temperature rising even two or three degrees centigrade. The blood vessels of the ears, on the contrary, quickly become constricted under the influence of the salts, and the ears appear white and quite cold.

The storage power of the individual organs is somewhat peculiar. The spinal cord and brain, in the majority of the experiments, stand first in their power of picking up and retaining the nickel and cobalt. This is in close accord with what has already been found with soluble forms of arsenic,* and more recently with strychnine sulphate.† It would have been interesting in this connection to have seen whether, as with arsenic, the form of the poison modifies the relative amount absorbed by the brain and spinal cord, but this we did not have time to try. Again, as with soluble forms of arsenic, the muscle tissue shows in several of the experiments, as Nos. V and VI, a marked affinity for nickel and cobalt, retaining a larger percentage of the metals than either the liver or kidneys. Still more noticeable, in all of the experiments but one, is the much larger amount of poison in the muscles of the back than in the muscles of the leg: a constant difference occurring in a tissue of the same kind, and hardly explainable on the ground of difference in vascularity. A somewhat similar distribution of arsenic in the muscle tissue was found by one of us a few years ago in an arsenical poison case.‡

* Chittenden, Amer. Chem. Journal, vol. v, p. 8, also Studies from Laboratory of Physiological Chemistry, vol. i, p. 141. Scolosuboff, Bulletin de la Société Chimique de Paris, vol. xxiv, p. 125.

† R. W. Lovett, Journal of Physiology, vol. ix, p. 99.

‡ Chittenden, Amer. Chem. Journal, vol. v, p. 12.

Another striking feature in the storage of nickel and cobalt by the tissues, is the comparatively large amount retained by the lungs and heart, the amount found in these organs generally exceeding the amounts stored up in the liver and kidneys.

## X.—Results obtained by Etching a Sphere and Crystals of Quartz with Hydrofluoric Acid. By Dr. Otto Meyer and Samuel L. Penfield.

A few years ago one of us* published the results of an experiment of etching a sphere of calcite with acetic acid in which the symmetry of a calcite crystal was brought out by the character of the etchings on the sphere and the final result of eating away the greater part of the calcite was a crystalline figure with rounded faces, but with a decided steep scalenohedral habit with truncations at the extremities of the vertical axis. This suggested to us the idea of trying similar experiments on spheres cut from other crystals. The difficulty of course lies in obtaining spheres of perfectly pure homogeneous material; the results furnish, however, an interesting and instructive means of studying the symmetry of any crystalline substance and as parts of the sphere are parallel to all possible faces of a crystal, as soon as the relation of the sphere to the axes of the crystal is made out the character of the etchings in any particular part of the sphere will determine the character of the etching produced by the solvent on any crystal face parallel to that particular part of the sphere. The ease with which spheres of Japanese quartz can be obtained and the readiness with which quartz yields in certain directions, to the action of hydrofluoric acid, made the following experiments quite easy, while the results as will be seen are far more striking than one would at first suppose.

The results of our experiments will be better understood by reviewing some experiments made in 1855 by F. Leydolt † on quartz crystals in which he showed that hydrofluoric acid acts very unequally on the different kinds of faces, so that not only the right and left-handed character of the crystals, but also all the complexity of twinning can be made to appear by etching. The experiments were repeated by us by placing simple quartz crystals from Herkimer, N. Y., in strong hydrofluoric acid and leaving them till sufficiently distinct etchings were produced. In these experiments, some of which were carried on in cold and some in hot acid, the character of

* Meyer, Jahrb. Min., 1883, i, 74.

† Sitz-ber. der Wiener Akad., 1855, xv, p. 59.

the etching was in all cases the same, and as quartz is dissolved by the acid very slowly it is not probable that slight changes in the temperature or strength of the acid would have made any appreciable difference. On the ordinary quartz combination of prism, *m*, I, $10\bar{1}0$, positive rhombohedron *r*, 1, $10\bar{1}1$ and negative rhombohedron *z*,–1, $01\bar{1}1$ the following etchings are very easily developed. The positive rhombohedron *r* yields most readily to the action of the acid becoming covered with elongated unsymmetrical depressions having a horizontal direction, the heaviest part being to the right in a right handed crystal, fig. 1, plate I, and to the left in a left handed crystal fig. 2, plate I. The top and middle edges of these depressions are nearly straight, the bottom slightly curved, the widest end is terminated by a straight edge having the direction of the zonal edge between *r* and the adjacent *z* face. These etchings are distributed thickly over the *r* faces, and although they are not all exactly alike, their general character is well represented in figs. 1 and 2. The effect of this action is also to eat away and replace all of the edges of the crystal toward which the heaviest ends of the etchings are turned; thus in a right-handed crystal between *r* and *r* ($10\bar{1}1$ and $\bar{1}101$), *r* and *z* ($10\bar{1}1$ and $01\bar{1}1$) and *r* and *m* ($10\bar{1}1$ and $01\bar{1}0$) all to the right, while the corresponding edges to the left toward which the points of the depressions on *r* are turned, are left perfectly sharp, except of course the upper parts where *r*, $10\bar{1}1$ forms a short edge with the adjacent *r*, $0\bar{1}11$ face to the left. In a left-handed crystal, this same phenomena can be observed only with the corresponding edges eaten away to the left instead of to the right. This replacement of the edges is not shown in figs. 1 and 2, but is shown in the original figures of Leydolt, who also determined the symbols of the faces replacing the different edges. According to our experience the replacement of the edges appears more like an accumulation of little facets, all reflecting the light simultaneously, than a replacement made by a single face and for a discussion of the symbols of the faces and the determination of the twinning structure of quartz as shown by the etchings we refer our readers to the original paper of Leydolt. If the crystals are left in the acid for a sufficiently long time the edges between the rhombohedron faces become so far eaten away that nothing is left of the original rhombohedron faces and the prism is left terminated by the etching faces alone, which flatten out the crystal very much in the direction of the vertical axis.

On the negative rhombohedron *z*, the etchings are of an entirely different character, composed of a system of shallow depressions

with curved contours, giving a sort of feather-like marking with the direction of greatest action turned toward the heaviest etching on the positive rhombohedron, figs. 1 and 2, plate I.*

The prismatic faces are much less acted upon than the rhombohedron faces, the etchings varying somewhat in character but consisting essentially of four sided depressions with long and short vertical edges parallel to the edges of the prism, one straight steep edge on the side of the positive rhombohedron *r* and parallel to the zonal edge between *m* and *z*, and a shorter slightly curved edge on the side of the negative rhombohedron *z*. These etchings have definite relations to the symmetry of the crystals and are of reverse character on right, fig. 1, plate I, and left, fig. 2, plate I, handed crystals. On adjacent prismatic faces, the longer or shorter vertical edges are turned toward each other, and by prolonged etching the alternating prismatic edges, toward which the shorter vertical edges of the etchings are directed, are slightly eaten away while the other prismatic edges remain sharp and perfect.

From a consideration of the above we can now more readily understand the action of hydrofluoric acid on a sphere cut from a simple quartz crystal. A sphere of about 2·44 c. m. diameter was purchased in New York, and etched by placing it in a lead crucible containing rather a strong commercial hydrofluoric acid, such as can be bought in rubber bottles from dealers in chemicals. The exact strength of the acid was not determined. No special care was taken to place the sphere in any particular position in the acid, its position being accidentally changed nearly every day when the acid was removed, and the solution of the quartz going on so slowly that the acid had a chance to act apparently equally on all similar parts of the sphere. During the progress of the etching, which was carried on slowly in the cold, photographs of the etched sphere were obtained at three stages, which seemed well suited for illustration.

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* According to my experience these etchings on the rhombohedron faces furnish one of the best methods of showing to a beginner in crystallography that the six faces which usually terminate a quartz crystal, are not the faces of an hexagonal pyramid, and all alike, but are those of a positive and negative rhombohedron. To prepare sections for showing this with a microscope, crystals should be etched till the markings are sufficiently distinct, then by cementing the crystal, with the etched face down, to a glass plate with Canada balsam and cementing glass plates on either side, the quartz can be ground away with emery till the glass plates form a large wearing surface and the quartz is ground to just the thickness of the glass plates; then after removing the slice of quartz and cleaning it, it can be cemented to an object glass with the etched surface up and is ready for examination with the microscope.—Penfield.

After leaving the sphere in the acid for a few hours, the etchings were distinctly observed and their arrangement on the sphere was such that its crystalline nature and relation to hexagonal axes could be determined. The location of the extremities of the vertical axis was marked by the centers of two triangular patches on opposite sides of the sphere, while the character and arrangement of the prominent etchings on the positive rhombohedron indicated the right handed character of the crystal from which the sphere was cut as well as the location of the extremities of the lateral axes. After being in the acid for about four days some of the etchings were very prominent, and the sphere had the appearance represented in figures 1 and 2, plate II. In figure 1 we are looking down upon the sphere in the direction of the vertical axis. In the centre there is a distinct, somewhat hexagonal field, the center of which marks the extremity of the vertical axis. This whole portion is one where the etching has gone on very vigorously, and with the microscope it can be seen that the surface is composed of minute triangular pyramids grouped closely together. About this, three prominent parts, which are arranged in the alternating sections of the hexagon, indicate the position of the positive rhombohedron by the greater extent of the etching, leaving very distinct prominences with their steep sides turned to the right. A distinct ridge or marking, from which the lines of etching go off very distinctly, can also be seen about in the center of each negative rhombohedron. In fig. 2, we are looking at the sphere about at right angles to a prismatic face. A little above the center of the figure and trending to the right, the prominent etchings, indicating the position of the positive rhombohedron, can be seen, while below and to the right they can also be seen in the position of the lower positive rhombohedron. On what may be called the equator of the sphere, midway between the above mentioned prominent etchings on the positive rhombohedrons above and below, the extremity of one of the lateral axes can be located a little to the right of the center of the figure. On much of the surface near the equator of the sphere, the original polish has not been destroyed. The vigorous action of the acid at the extremities of the vertical axis is plainly seen accompanied already by a slight flattening of the sphere.

After exposing the sphere again to the action of the acid for about two weeks it had the appearance represented in figures 3 and 4, plate II. In figure 3, where we are looking down upon the sphere in the direction of the vertical axis, three parts on the equator, located by

the right hand and upper and lower left hand angles of the hexagon, indicate one extremity of each of the three lateral axes and from these parts the lines of etching run out very beautifully toward the center and the prominent marking on the rhombohedron faces. In figure 4, where we are looking at right angles to the vertical axis, besides the decided flattening, a rhombic portion, about in the center of the field, is conspicuous, the center of which locates the extremity of one of the lateral axes. On this portion not only could the original curved surface of the sphere be detected but also the original polish. The acid having had apparently no action on this portion of the sphere, while the etched portions come up to meet this with sharp and distinct angles. Owing to a slight misunderstanding a mistake was made in photographing figure 4, which was not discovered till it was too late to correct it. If we imagine the sphere turned 90° so that the unattacked portion would appear at the right and seen at a tangent, while one of the two similar portions which are now behind and out of sight would appear in the front and a little to the left, the quartz would appear in just the right position to compare with figures 2 and 6. As it is we are looking at the crystal not at right angles to a prism *m* but at right angles to a prism of the second order $\bar{1}2\bar{1}0$.

By exposing the quartz for about one month longer to the action of the acid it appeared as represented in figures 5 and 6, plate II. In figure 5, which is again a vertical view, we can readily locate the extremities of the three lateral axes by the right hand and upper and lower left hand angles of the hexagon. At these parts the curved contour of the sphere is preserved for a short distance, but between them there is a decided tendency toward a triangular cross section. The sphere as will be seen from figure 6 has become extremely flattened and the upper and lower portions meet along a very sharply defined line. The etchings seem to arrange themselves along parallel lines or ridges and some idea of their beautiful arrangement can be obtained from the larger reproduction shown in figure 3, plate I. In figure 6 we notice, in addition to the extreme flattening, two of the three portions where the acid has had very little action, one taken at a tangent to the right, the other a little to the left of the center; these appear as very conspicuous parallelograms; they have a curved surface similar to that of the original sphere, and although the original polish has disappeared from them only the finest etchings can be detected with the microscope. It can almost be said that the acid has had no action on these three surfaces, at least not enough to

destroy the original polish of the sphere till toward the very end of the experiment and not enough to appreciably diminish the diameter of the sphere. Although the original diameter was not accurately measured care was taken soon after commencing the etching to cut a hole in a card board very carefully just large enough to allow the sphere to pass and at the conclusion of the etching the quartz just touched at these three points when passed through the same hole.

The quartz was still exposed to the action of this acid for about a week, but the general effect was not different from that shown in figures 5 and 6. Of course the sphere was still further flattened in the direction of the vertical axis and the three parts at the extremities of the lateral axes where the acid had acted least, became considerably changed, being flattened out more in a vertical direction and therefore appearing as parallelograms, relatively much more elongated horizontally. Figure 3, plate I, is from a photograph taken at this stage in which the relation of these parts to the longer sharp edge between them is less than in figure 5, plate II. At this stage also the etching of the sphere was stopped and the specimen deposited in the collection of Professor George J. Brush, New Haven, Conn.

In review it will be noticed, as is the case in all etching, that the acid acts very unequally on different faces of a crystal and therefore on different parts of the sphere, equally, however, on those similar parts of the sphere which are similarly situated with reference to hexagonal axes. The action is greatest at the two extremities of the vertical axis. The action seems to be, especially toward the end, to lift off or dissolve away layers of molecules from above and below while there are three parts, which are each one of the ends of three lateral axes, where the acid exerts practically no solvent action. These parts diminish in size as the action of the acid continues but not by any action of the acid upon them directly, except as the molecules were taken away from above, below and at the sides. This is one of the most striking features of the experiment that in these three directions quartz is almost absolutely insoluble in hydrofluoric acid. As a study in symmetry the experiment all along was a very interesting one. The etched sphere could never be divided by a plane into symmetrical halves and showed throughout all of the experiment the tetratohedral character of a right-handed quartz crystal. The sphere was cut from a crystal which would have shown etchings like those in figure 1, plate I. The accompanying illustrations give only a faint idea of the beauty of the etched sphere, it

being impossible to reproduce the delicacy and beauty of the markings as they appeared on the perfectly transparent material of the quartz.

*Pyro-electrical experiments.*—To further test the relation of the sphere to crystallographic axes, it was heated for some hours in an air bath to 100° C. and on cooling dusted with a mixture of red oxide of lead and sulphur, according to the excellent method described by Professor A. Kundt of Strassburg.* The red oxide of lead and yellow sulphur arranged themselves in six alternating vertical bands about the equator of the sphere, the red bands being located midway between the heaviest parts of the etchings on the positive rhombohedrons above and below. On the etched sphere represented in figure 2, plate II, a red band ran vertically a little to the right of the center, a yellow one a little to the left of the center and so on, three red alternating with three yellow about the equator of the sphere. On the etched sphere represented in figures 5 and 6, plate II, the slightly attacked parallelogram parts, representing the ends of the lateral axes, were yellow while the center of the sharp edges midway between them were red. These pyro-electrical phenomena, according to B. von Kolenko,† indicate that the crystals from which our sphere was cut was a right-handed one; that part of the sphere where negative electricity develops on cooling and where the positively electrified red oxide of lead deposits, indicating the position of the prismatic edge, where the right-handed trapezohedral faces above and below would occur. The even distribution of the pyro-electricity into six alternating positive and negative sections prove the simple character of the sphere and the absence of twinning. This latter was very important for the success of our experiment, for if the sphere had been cut from a complicated twin crystal the etchings would have arranged themselves in a very confused manner, and the shapes produced by the etching would have been very much modified.

In closing we wish to express our thanks to Mr. J. M. Blake of New Haven, for the care which he took in photographing such a difficult, transparent object, and to Mr. E. Bierstadt of New York, for the pains which he took in the preparation of the plates.

Mineralogical Laboratory, Sheffield Scientific School, Feb. 4th, 1889.

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* Ann. d. Phys. u. Chem., 1883, xx, p. 592.

† Zeitsch. f. Kryst., ix, 1884, p. 1.

## EXPLANATION OF PLATES.

### Plate I.

1. Etchings produced on the faces of a right handed quartz crystal by hydrofluoric acid.

2. Etchings produced on the faces of a left handed quartz crystal by hydrofluoric acid.

3. Final result of eating away the greater part of a sphere (cut from a right handed quartz crystal) with hydrofluoric acid during a period of about eight weeks. Seen in the direction of the vertical axis. The angles of the hexagon mark the extremities of the lateral axes.

### Plate II.

1 and 2. Appearance of the etched sphere after being in the acid about four days. 1. Seen in the direction of the vertical axis. 2. Seen in the direction at right angles to the vertical axis and a prism of the first order.

3 and 4. Appearance of the etched sphere after being in the acid about three weeks. 3. Seen as in 1. 4. Owing to a mistake in taking the photograph seen about at right angles to a prism of the second order.

5 and 6. Appearance of the etched sphere after being in the acid about seven weeks. 5. Seen as in 1 and 3. 6. Seen as in 2.

## XI.—New England Spiders of the Families Drassidæ, Agalenidæ and Dysderidæ. By J. H. Emerton.

### Drassidæ.

The *Drassidæ* have long bodies like the *Agalenidæ* and *Lycosidæ*, but most of them are a little flattened above and walk with their bodies near the ground; the first and second feet are directed forward and the third and fourth backward. The feet have but two claws, under which is usually a cluster of hairs sometimes so thick as to conceal the claws. The under sides of the tarsus and metatarsus are sometimes covered with hairs, especially on the first and second legs, and these hairs are often flattened or thickened at the end. The cephalothorax is low in front, the highest part being in the middle or farther back. The eyes are in two nearly straight rows. The spinnerets are cylindrical with the tubes on the end, and the upper and under pairs are nearly equal in length.

None of the *Drassidæ* make webs for catching insects, though many of them make nests, usually flattened tubes, in which they hide in winter or while moulting or laying eggs. Most of them live on the ground and hide under leaves and stones. A few, as *Clubiona*, *Chiracanthium*, and *Anyphæna* live in summer on plants several feet above the ground.

Many species are found adult at all seasons and probably live several years.

The cocoons are flat, some are attached by one side, but most of them lie loose in the nest or hiding place.

A large number of American species were described and figured by Hentz, most of them under the generic name *Herpyllus*. Of these I have identified nine: *H. descriptus*, *crocatus*, *alarius*, *ecclesiasticus*, *ater*, *bilineatus*, *variegatus*, and *Clubiona saltabunda*, *pallens*. Several others have been described by Thorell, and of these I have identified *Gnaphosa brumalis* Thor., Proc. Boston Soc. Nat. Hist., vol. xvii, 1875, and *Gnaphosa conspersa*, *G. scudderi*, and *Prosthesima melancholia* Thor., Bulletin Hayden's U. S. Geol. Survey, vol. iii, No. 2, 1877. The specimens of this family in the Museum of Comparative Zoology in Cambridge, Mass., have been named by Keyserling, and I have adopted his names for several species though they are not yet published.

## Micaria Westring.

This genus was first separated from *Drassus* by C. Koch under the name of *Macaria*, which had before been used for a genus of *Lepidoptera*, and was therefore changed by Westring to *Micaria* in 1851. They are all small and slender spiders with the cephalothorax arched upward in the middle, without any dorsal groove or only a thick opaque spot in place of it. The abdomen and usually the cephalothorax are covered with flattened scales sometimes brightly colored and iridescent. The tarsus and metatarsus of the first and second feet have a double row of flattened hairs on the under side.

Hentz's *Herpyllus auratus*, found farther south, belongs to this genus and is nearly related to *M. longipes*.

### Micaria longipes, new sp.

PLATE III, FIGURES 1*a*, 1*b*, 1*c*, 1*d*, 1*e*, 1*f*, 1*h*.

Largest specimen $5^{mm}$ long. Pl. III, fig. 1. The cephalothorax is twice as long as wide, widest across the middle. Head not much narrowed. The eyes occupy half the width of the head. The front row is nearly straight, the upper row with the middle eyes highest. Eyes all nearly of the same size. The cephalothorax is highest in the middle, curving downward toward both ends. The abdomen is one-half longer than the cephalothorax and about as wide, blunt at both ends and drawn in a little at the sides and above about one-third its length from the front. The legs are long and slender, the fourth pair longest. The colors are light yellowish brown with gray hairs and scales which have green and red metallic reflections on the abdomen. The legs are darker from before backward, the front pair all light yellow except the femur, and the fourth and third pairs with longitudinal brown stripes that nearly cover the tarsal joints. The cephalothorax is without markings. The abdomen has a pair of white stripes at the constricted spot and a less distinct pair near the front end. At the hinder end it is almost black. The scales of the abdomen are of various forms, those of the white spot are long with several branches at the base figs. 1*d*, 1*e*, those of the front of the abdomen are more simple, fig. 1*c*, and those behind the white markings are half as wide as long with two rows of short branches fig. 1*f* at some distance from their edges. The under side of the abdomen is as dark as the upper side. The white markings extend under half way to the middle line. The sternum is nearly twice as long as wide, widest at the second pair of legs and narrowed to a point behind.

The maxillæ are a little notched on the outer sides and straight on the ends except at the inner corners. The labium is two-thirds as long as the maxillæ. It is narrowed toward the tip, where it is about half as wide as at the base, fig. 1*a*.

The male palpi are small, the patella and tibia are about of the same length; at the base of the tibia on the upper side is a large tooth nearly as long as the diameter of the palpus, and curved strongly forward, fig. 1*b*. The tarsus is as long as the tibia and patella together, and pointed at the end. The palpal organ is small, fig. 1*b*.

The epigynum of a female from Salem, Mass., appears as in fig. 1*h* with two oblique openings near the posterior edge.

Salem and Medford, Mass., under stones and leaves. Adult male in August and adult female in June.

## Micaria montana, new sp.

PLATE III, FIGURES 2, 2*a*.

This is smaller than the common species. A female measures $4^{mm}$ long. The cephalothorax is not twice as long as wide and the widest part is behind the middle. The abdomen is twice as long as wide, not constricted or truncated at either end. The cephalothorax and legs are light yellow-brown, the legs lighter toward the ends. The abdomen is greenish brown with iridescent scales. Across the middle is a distinct narrow white line and a less distinct one crosses the front of the abdomen. On the hinder half of the abdomen are four or five white spots. The white markings extend a short way under the abdomen. The epigynum, Plate III, fig. 2*a*, has two oblique openings near the posterior edge turned more toward each other and less downward than in *Micaria longipes*.

Mt. Washington, N. H., July 1, 1874, east side, near the Ledge.

## Geotrecha, new genus.

This genus includes a number of American spiders described by Hentz, under the name of *Herpyllus*. Besides *Herpyllus descriptus* and *crocatus*, *H. ornatus*, *H. longipalpus*, *H. marmoratus*, *H. cruciger*, *H. conarius*, *H. trilineatus*, probably belong in it. *H. descriptus* and *H. crocatus* were placed by Koch in the genus *Agrœca*, with which they agree in the shape of the maxillæ and position of the eyes. In this, he was followed by Keyserling, who named the specimens of that species in the Museum of Comparative Zoology, in Cambridge, Mass., *Agrœca crocata*. In the same collec-

tion another species, *Geotrecha bivittata*, is named by Keyserling *Castianeira bivittata*, the genus *Castianeira* having been named by Keyserling, in 1879, for a South American spider, with a long, slender cephalothorax and a slender abdomen with the front part hardened and differently colored from the softer part.

In our species of *Geotrecha*, the cephalothorax is about two-thirds as wide as long and narrowed in front, more in some species than in others. The abdomen is longer and a little wider than the cephalothorax. It sometimes has a small, hard patch at the front end which is of the same color as the rest of the back and not easily seen. The abdomen is round, not flattened above as it usually is in *Prosthesima*. The legs are long and slender. The hairs on the under side of the first and second legs are only slightly flattened and thickened, and the claws concealed by a thick bunch of hairs. The maxillæ are nearly straight as in *Agræca* and the labium is as short as wide. The eyes are close together in the middle of the front of the head, the front row nearly straight and the hind row with the middle eyes highest. The middle eyes of both rows are largest and farther apart than they are from the lateral eyes. The spinnerets are very small and close together. The colors are dark brown and black, with white or bright colored markings.

The male palpi have the patella and tibia both short and the tarsus long and tapering. The palpal organ is round at the base and tapers to a fine point. The epigynum has two simple openings directed backward, and differing in size and distance apart in different species.

## Geotrecha bivittata.

*Castianeira bivittata* Keyserling, specimens in Cambridge Museum.

PLATE III, FIGURES 3*a*, 3*b*, 3*c*, 3*d*.

Length, 7 or 8$^{mm}$. Legs of fourth pair, 10 or 11$^{mm}$. The cephalothorax is widest across the middle in front of the dorsal groove, Plate III, fig. 3*a*, and is about half as wide at either the front or hinder end. The abdomen is usually about as long as the cephalothorax and widest at the hinder third. It is sometimes slightly drawn in at the sides and above over the front white marking. This is caused by contraction in alcohol, the front end of the abdomen being hardest contracts less than that part just behind it. The legs are long and tapering, the fourth pair longest.

The cephalothorax is dark brown. The abdomen is of the same color, a little lighter, with two white cross stripes, one about the middle of the back and the other, a less distinct one, farther for-

ward. The femora of all the legs are striped lengthwise with brown and yellow. The hind legs are brown with a little yellow on the upper side of the patella and tibia. The other legs are yellow, sometimes with brown stripes on the under side. The white marks on the abdomen extend underneath half way to the middle line. The front hard part of the under side of the abdomen is lighter than the hinder part, and the sternum is of the same color. The coxæ are lighter yellowish brown.

The epigynum shows through the skin as three dark spots and has two openings directed backward, fig. 3*d*.

The male palpi have the patella very short, about half as long as the tibia, fig. 3*b*. The tarsus is very large and dark colored, wide at the base and tapering toward the tip. The palpal organ is similarly shaped, with a rounded bulb through which the coiled tube can be seen, and a slender tip lying in a groove in the tarsus, fig. 3*c*.

Hentz's *Herpyllus zonarius* and *trilineatus* seem to be near this species.

It lives under leaves at all seasons of the year, and though not so quick in its motions as *crocata* is a difficult spider to catch except in cold weather, when it is often sifted from leaves in a torpid condition.

Massachusetts, Connecticut, and in N. Pike's Long Island collection.

The color is sometimes lighter, the whole cephalothorax above and below being light orange color, and the legs the same color, with the longitudinal brown stripes very narrow and indistinct.

In young individuals of both varieties the sternum is wider and more convex than in adults.

## Geotrecha pinnata, new sp.

PLATE III, FIGURES 4, 4*a*.

The largest specimen is $7^{mm}$ long, cephalothorax, $3^{mm}$.

The cephalothorax is shaped much as in *C. crocata*. The abdomen is oval, not so much narrowed in front as in the other species.

The cephalothorax is light brown. The abdomen is grayish brown with several white transverse stripes. The two widest stripes are in the same position as the two stripes of *C. bivittata*. On the front of the abdomen is another stripe, and on the posterior half are four or five others, some of them incomplete on the middle of the back. The femora of all the legs are light brown, the first, second and third legs are yellow, except the femora. The fourth legs have the tarsus and the ends of the tibia yellow, the rest light brown. Plate III, fig. 4.

Epigynum, like that of *crocata*, but with the holes larger and nearer together, fig. 4*a*.

The palpal organs and male palpi resemble those of *crocata*, but are a little larger.

Three specimens, of different ages, from Medford, Mass., under leaves with *C. bivittata*. Three adult females from Topsfield, Mass., Sept. 3d, under log in woods. Males and females in N. Pike's Long Island collection.

## Geotrecha crocata.

*Agræca crocata* Keys., specimens in Mus. Comp. Zool, Cambridge, Mass.

♀ *Herpyllus descriptus* Hentz.

♂ *Herpyllus crocatus* Hentz.

PLATE III, FIGURES 3*b*, 3*c*, 3*d*.

Length of female, 8 to 10$^{mm}$; cephalothorax, 4$^{mm}$. The cephalothorax is nearly twice as long as wide and widest across the dorsal groove. It is not narrowed behind as much as in *C. bivittata*. The abdomen is usually longer than the cephalothorax and a little wider at the widest part.

The cephalothorax is very dark brown or black, and the femora and coxæ of all the legs are the same color. The abdomen is black with a bright red spot of variable shape and size at the posterior end. The spot turns yellow in alcohol. In some specimens it is wanting. In the males the red spot is usually larger, sometimes extending the whole length of the abdomen. The hind legs are black or brown their whole length, a little lighter at the ends; the other legs are yellow, except the femora. The under side of the body is all black.

The epigynum has two small round openings, wide apart, a little in front of the transverse fold. Plate III, fig. 3*d*.

The male palpi are much like those of *C. bivittata*, but the tarsus and palpal organ are only about half as large and the patella and tibia are nearly equal in length. The tibia has a short process on the under side. Figs. 3*b*, 3*c*.

This spider lives among stones in dry, open places. It is easily alarmed and moves very rapidly. The flat, parchment-like cocoons common on stones in pastures are probably made by this species.

Massachusetts and Connecticut, and in N. Pike's Long Island collection.

## Prosthesima L. Koch.

Cephalothorax widest in the middle and more than half as wide in front. Eyes near together, occupying about half the width of the head. The middle eyes of both rows smaller than the lateral and nearer together than they are to the lateral eyes. Upper row straight, or the lateral eyes a little farther back. Sternum large and nearly as wide as long. Maxillæ wide in the middle and but little widened at the ends, Pl. III, fig. 6*a*. First and second legs with flattened hairs under the tarsus and part of the metatarsus.

### Prosthesima atra.

*Herpyllus ater* Hentz.
*Prosthesima funesta* Keyserling, specimens in Mus. Comp. Zool., Cambridge, Mass.
*Prosthesima melancholica* Thorell, Bull. Hayden's U. S. Geol. Survey, Vol. III, 1877.

PLATE III, FIGURES 6, 6*c*, 6*d*.

Female, 8$^{mm}$ long; cephalothorax, 3$^{mm}$. Male smaller. Cephalothorax and abdomen both a little flattened above. Cephalothorax narrow in front, about half as wide as in the middle. Plate III, fig. 6. Abdomen oval, the hinder half usually a little wider than the front. Sternum very large, almost as wide as long, fig. 6*a*. Maxillæ and labium large and a little shorter and wider than in *P. ecclesiastica*. Feet 1 and 2 with flattened hairs under the tarsus and half the metatarsus. Feet 3 and 4 with fine hairs in the same places.

The whole body is black in most individuals, sometimes, especially in the young, yellowish brown on the ends of the feet and under the abdomen.

Epigynum large and distinct with two small depressions in front and large openings behind surrounded by a thick brown rim, fig. 6*d*.

The male palpus is short with a very large tursus, as long as the tibia and patella together and more than half as wide. The process on the outside of the tibia is about as long as the tibia itself and nearly straight. The palpal organ has a small fine tube and several small hooks and processes all at the tip end of the palpus, fig. 6*c*.

This spider lives under stones and leaves. The cocoon is flat on one side, by which it is attached, and convex on the other. It is white, or sometimes a little pink.

Mt. Washington, N. H., Eastport, Me., Massachusetts, and in N. Pike's Long Island, N. Y., collection.

*P. melancholica* was found by Dr. A. S. Packard at Monitou, Colorado, 1875.

**Prosthesima depressa,** new sp.

PLATE III, FIGURES 8, 8*a*.

A smaller species than *atra*. Female, 6$^{mm}$ long. The head is much smaller than in *atra* and the eyes larger and closer together. Plate III, fig. 8. The cephalothorax and abdomen are black. The first and second legs have the tarsus and metatarsus pale yellow, the rest of the legs black except a pale spot on the outside of each femur. The third and fourth legs have the tarsus and metatarsus pale, the tibia black at the distal end, the leg becoming lighter from this point to the base. Underneath, the coxæ are darker from behind forward.

The epigynum has the openings at the sides, farther forward, and the ridges over them thicker and shorter than in *atra*, and the two little depressions in front appear to be wanting, fig. 8*a*.

Medford, Mass., July 23.

**Prosthesima ecclesiastica.**

*Prosthesima propinqua* Keys.
*Herpyllus ecclesiasticus* Hentz.

PLATE III, FIGURE 7, 7*a*, 7*b*, 7*c*, 7*d*.

This spider is about 8 or 10$^{mm}$ long, a little smaller than *Gnaphosa conspersa* which it resembles in form and habits, but from which it is easily distinguished by its colors. The cephalothorax is black at the sides and has a whitish stripe in the middle. The abdomen is black at the sides with a bright white stripe in the middle that extends from the front end about two-thirds its length. At the hind end of the abdomen, just over the spinnerets, is another white spot. The legs are dull black turning to brown in alcohol, as does the cephalothorax. The under side of the abdomen is dark at the sides and light in the middle.

The eyes cover about half the width of the head, The two rows are nearly equal in length, the hinder only a little the longer. Seen from above, both rows appear straight. Pl. III, fig. 7.

The maxillæ are widened at the end, the outer corner sharp and the inner rounded off down to the lip. Fig. 7*a*.

The male is much smaller than the female but similarly marked. The male palpi are small. The patella and tibia are short, and the tarsus is as long as both together. The process on the tibia is slender and about equals the tibia in length. It is on the outer side, showing indistinctly from above. It is slightly forked at the tip. The

palpal organ is simple, with two short processes on the outer end. Fig. 7*c*, 7*d*.

The epigynum has a small oval opening at the posterior end of a dark area. Fig. 7*b*.

Under stones. Boston, Salem, Danvers, Wood's Holl, Mass.; Albany, N. Y.; Providence, R. I.

## Pœcilochroa Westr., Simon.

PLATE IV, FIGURES 1*a*, 3*a*.

The cephalothorax is narrowed toward the front, as in *Prosthesima*, and more narrowed in males than in females. The two rows of eyes are far apart, the hinder row a little longer than the front row, with the lateral eyes farther back than the middle ones. The middle eyes are farther apart than they are from the lateral eyes. The labium is not much longer than wide and a little narrowed toward the end. The maxillæ are about twice as long as the labium. They are narrower at the base and widen to the insertion of the palpi. From the palpi the maxillæ curve inward and nearly meet in front of the lip. The outer corners are turned outward. Pl. IV, figs. 1*a*, 3*a*. The colors are bright and the markings distinct.

### Pœcilochroa variegata.

*Herpyllus variegatus* Hentz.

*Drassus variegatus* Keyserling, specimens in Mus. Comp. Zool., Cambridge, Mass.

PLATE IV, FIGURES 1, 1*b*, 1*c*.

This is one of the most distinct and brightly colored species of the family. Pl. IV, fig. 1. The cephalothorax is bright orange, a little darker toward the eyes. The abdomen is black with three white transverse stripes from the middle of which a T-shaped white mark extends half way to the front stripe. On the front half of the abdomen the white stripes are usually partly colored with orange. The femora of the first and second legs are black. The distal end of the femur and both ends of the tibia of the fourth legs are black. The legs are otherwise orange colored. The hinder row of eyes is considerably longer than the front row, the rear lateral eyes being their diameter nearer the sides of the head than those of the front row. The head of the male is much narrower than that of the female. The male palpus has a process on the outer side of the tibia half as long as the tarsus, tapering toward the end and slightly bent inward at the tip. The tube ends near the outer end of the tarsus and is supported by a short thick process. Figs. 1*b*, 1*c*.

This spider is common under leaves in dry woods. Eastern Massachusetts; Dublin, N. H.; New Haven, Conn.

**Pœcilochroa montana,** new sp.

PLATE IV, FIGURES 2, 2*a*.

This species is a little larger and less brightly colored than *P. variegata.* Pl. IV, fig. 2. Female $8^{mm}$ long, cephalothorax $3.5^{mm}$. The arrangement of the eyes and proportions of the body are about the same. The cephalothorax and legs are dark brown, the hinder ones a little the lighter. The abdomen is black with a pair of white spots near the front end and another pair across the middle nearly united in the middle. The sternum and coxæ are dark brown. The epigynum is dark brown with a small opening at the hind end. Fig. 2*a*.

Mt. Washington, N. H., on the road to Gorham.

**Pœcilochroa bilineata.**

*Herpyllus bilineatus* Hentz.

PLATE IV, FIGURE 3, 3*a*.

A very distinct species on account of its markings. Cephalothorax white, with two black stripes and a fine black line on the edge, each side. Pl. IV, fig. 3. Abdomen thickly covered with long hairs, white in the middle and at the sides, and with two wide black stripes that do not extend quite to the end. The under side of the abdomen is white with a black stripe each side. Fig. 3*a*. The legs are covered with gray and white hairs.

The female is $7^{mm}$ long, cephalothorax $3^{mm}$. The head is about half as wide as the middle of the thorax. The eyes are small, the middle pair in each row farther apart than they are from the lateral eyes. The two rows are widely separated. Sternum oval, widest in the middle. Spinnerets long. Epigynum small, with a single opening directed backward just in front of the transverse fold.

## Gnaphosa Latr., 1804.

**Gnaphosa brumalis** Thorell, Proc. Boston Soc. Nat. Hist., vol. xvii, 1875.

*Gnaphosa scudderi* Thorell, Bull. Hayden's U. S. Geol. Survey, vol. iii, 1877.

PLATE IV, FIGURES 5, 5*a*, 5*b*.

This species is a little smaller than *G. conspersa.* A female of the usual size is $10^{mm}$ long, cephalothorax $4^{mm}$, while *conspersa* grows to the length of 12 or $15^{mm}$.

The colors are the same as those of *conspersa;* cephalothorax and legs dark brown and abdomen rusty black.

The epigynum has the openings rather wider apart, and the front middle appendage flat, wrinkled at the edges, and with a hard spot in which is a small hole near the end. This appendage resembles the finger in the same position in *Epeira.* Pl. IV, fig. 5*b*.

The male palpus has the tibia rather shorter and its outer process longer than in *conspersa,* fig. 5. The tube of the palpal organ is only about half as long, its base being nearer the middle of the tarsus. The middle hooked appendage is as long as in *conspersa,* but much more slender, fig. 5*a*.

Under stones on Mt. Washington, N. H., from the ledge upward, with cocoons of eggs July 1.

Males and females from Ellis Bay, Anticosti, July 23, S. Henshaw in collection of Boston Soc. Nat. Hist.; females with cocoons of eggs.

The specimen named by Thorell was from Strawberry Harbor, Labrador, collected by A. S. Packard in 1864.

*G. scudderi* was found by A. S. Packard at the Garden of the Gods, Colorado, in 1875.

**Gnaphosa conspersa** Thorell, Bull. Hayden's U. S. Geol. Survey, vol. iii, 1877.

*Gnaphosa gigantea* Keyserling, specimens in Mus. Comp. Zool., Cambridge, Mass.

PLATE IV, FIGURES 4, 4*a*, 4*b*, 4*c*, 4*d*, 4*e*.

This spider is 12 to 15$^{mm}$ long and rusty black in color. Some specimens freshly moulted are dull yellowish or greenish gray, and old individuals have a brownish color. The whole body and the legs are covered with long hairs. The cephalothorax is wide in front and the eyes are not so close together as in *Pythonissa imbecilla,* or in *Prosthesima.* The hinder row of eyes is a little longer than the front row, and the lateral eyes are larger and farther back than the middle ones. Pl. IV, fig. 4. The middle hinder eyes are a little oval and oblique, diverging toward the front. The mandibles are large and strong, on the inner side under the claw they have a wide flat tooth with irregular and serrated edge, and near the inner corner two large pointed teeth, fig. 4*b*. The maxillæ are very wide and curve inward so as nearly to meet around the end of the lip. Their outer corners are rounded, fig. 4*a*. The spinnerets are stout and the lower pair are widely separated. The male differs but little from the female. The male palpi have the patella and tarsus both short and the

tarsus as long as both of them. The tibia has a short pointed process extending forward over the tarsus, fig. 4*c*. The tarsus is curved outward at the end. The tube of the palpal organ is slender and extends along the outer edge of the tarsus for its whole length. At the outer end of the palpal organ is a short process flat at the end and curved downward, fig. 4*d*. The epigynum has a long opening on each side and a short transverse pit in front of them in the middle, fig. 4*e*. In western specimens the opening at the hinder part of the epigynum has the sides more nearly parallel, not diverging forward as much as in those from New England.

It lives under stones and leaves. The cocoon is white and flat, with its diameter as great or greater than the length of the spider. The female stays near the cocoon, but makes no nest.

All over New England, from the White Mountains, N. H., to New Haven, Connecticut; Adirondacks, N. Y.

Thorell's specimens were collected by A. S. Packard in 1875. A female with cocoon of eggs on Gray's Peak, Colorado, over 11,000 feet high, on fir, Kelso's cabin, Colorado, and a small one from Idaho, all adult females and all smaller than most eastern specimens.

## Pythonissa Koch.

**Pythonissa imbecilla** Keyserling, specimen from Kentucky in Mus. Comp. Zool., Cambridge, Mass.

PLATE IV, FIGURES 6*a*, 6*b*, 6*c*, 6*d*.

The male is about $4^{mm}$ long and the female $5^{mm}$. The two rows of eyes are nearly of the same length, the hinder row almost straight, with the lateral eyes only slightly farther back than the middle ones. The lateral eyes of both rows are larger than the middle eyes. The maxillæ are almost as wide as long and are curved inward so as to touch in front of the lip. Pl. IV, fig. 6*a*. The front edge of the maxillæ is straight with the corners only slightly rounded. The mandibles are small and the wide tooth under the claw, fig. 6*b*, can be plainly seen just in front of the maxillæ with another pointed tooth on its inner side. The cephalothorax, both above and below, and the legs are orange-brown with black hairs. The abdomen is bluish black with a few whitish hairs at the hind end and around the four muscular spots near the middle. The epigynum, as in fig. 6*d*. The patella and tibia of the male palpus are very short and not so thick as the femur. The tarsus is as long as the patella and tibia together. The palpal organ is large and complicated, the tube showing plainly across the outer end, fig. 6*c*.

Under stones. Males from Dublin, N. H., and Dedham, Mass. Females from Bluehill, Milton, Mass.

## Drassus.

PLATE IV, FIGURE 7*a*.

The genus *Drassus* of Walckenaer included the greater part of the present family *Drassidæ*, as well as some *Agalenidæ* and *Cinifionidæ*. From this, various genera have been separated from time to time, leaving the present *Drassus* an ill defined group containing species differing greatly among themselves and forming several groups, which further study will no doubt make it possible to separate. The only two species which I place in this genus belong, one near the European *D. lapidosus* and the other near *D. troglodytes*. In these species the cephalothorax is wider in front and less flattened than in *Gnaphosa* and *Prosthesima*. The eyes are small and separated by spaces at least as wide as their diameter. The front row is nearly straight. The posterior row is longer, and curved with the lateral eyes lower than the middle. The middle hinder eyes are oval and turned apart toward the front, and are nearer together than to the lateral eyes. The mandibles and maxillæ are large and stout. The maxillæ are widened on both sides beyond the insertion of the palpi, the outer corners are slightly rounded and the inner corners slope obliquely toward the lip. Pl. IV, fig. 7 *a*. The lip is about half as long as the maxillæ. The colors are gray and drab with fine short, white or gray hairs, and only faint markings on the abdomen.

### Drassus saccatus, new sp.

PLATE IV, FIGURES 7, 7*c*, 7*d*.

This is one of the most common of our *Drassidæ*. Pl. IV, fig. 7. The female grows to be $12^{mm}$ long, with legs $10^{mm}$ to $15^{mm}$. The color is light gray sometimes with indistinct transverse dark markings on the abdomen. The color of the front part of the head is a little darker and the feet and mandibles and maxillæ are brown. The abdomen is long and slender as in *Clubiona*. The epigynum is small and has two dark round depressions just in front of the fold, fig. 7*d*.

The male is smaller and more slender. The male palpi are very long the patella, and tibia are together as long as the femur, and all are as long as the femur of the first legs. The tibia has a small process on the outer side, fig. 7*c*. The tarsus is long and narrow,

and the palpal organ is small with a short tube near the distal end, fig. 7*c*.

These spiders live under stones in a large bag of silk in which the female stays with her cocoon of eggs. In the early summer a male and female live together in the nest, the female often being immature.

White Mountains, N. H., to Connecticut.

**Drassus robustus**, new sp.

PLATE IV, FIGURES 8, 8*a*, 8*b*, 8*c*.

This is a smaller and shorter legged species than the preceding. Pl. IV, fig. 8. The female is $8^{mm}$ long. The colors are darker and redder, especially toward the head. The sternum, maxillæ and mandibles are dark brown. The head is as wide as in *D. saccatus*, and the eyes are a little closer. The epigynum is large, light colored in the middle, and with a dark ridge each side, fig. 8*a*. The male is much smaller than the female. The palpal organs and tarsi are large and round. The tarsus is short and has a short curved process that extends over the tarsus on the upper side, fig. 8*b*, 8*c*.

Medford, Mass., July.

## Clubiona Latr.

Cephalothorax very wide in front. Front row of eyes straight, or with the middle pair a little higher than the lateral, nearly equal in size and equidistant, or the middle a little larger or farther apart than they are from the lateral. Upper line of eyes longer and slightly curved with the middle pair highest, the eyes all larger than those of the front row, and the middle pair usually farthest apart. Plate V, fig. 8*c*. Maxillæ long, narrow at the base, and much widened beyond the insertion of the palpi. Fig. 10. The mandibles are stout and convex at the base in the females. In the males the mandibles are more slender, longer and tapering at the tips, sometimes with sharp ridges along the sides. Legs slender, the fourth pair longest. Feet with long claws, the first and second pairs with the under side of tarsus and metatarsus covered thickly with hairs widened at the end. The abdomen is truncated in front and tapering behind. The colors are always pale gray and drab, usually with darker brown on the head around the eyes, and rarely a light brown or gray pattern on the back of the abdomen. The body is covered with short and fine hairs which give it a soft silky appearance without concealing the color of the skin.

Most species vary greatly in size, some mature individuals being twice as large as others of the same age and sex. They live on plants in summer, and in winter hide under bark or stones, and have at all seasons flat tubular nests of silk.

**Clubiona crassipalpis** Keyserling, specimens in Mus. Comp. Zool., Cambridge, Mass.

PLATE V, FIGURES 1, 1*a*, 1*b*.

Male 8$^{mm}$ long, sometimes smaller. Cephalothorax two-thirds as wide as long. Male mandibles convex in front for two-thirds their length, with a low ridge along the inner side and around the end of the convex portion. The thin keel on the front outer edge is sharp but short, extending only a little over the convex part.

The tibia of the male palpus is as long as the patella. The process on the outer side is long and slender, the end curved inward over the tarsus. Plate v, figs. 1, 1*a*, 1*b*.

Abdomen marked with brown irregular veins, the rest of the body pale. Head a little darker toward the front.

Massachusetts, Connecticut, Albany, N. Y., Providence, R. I., and in N. Pike's Long Island collection.

**Clubiona mixta**, new sp.

PLATE V, FIGURES 2*a*, 2*b*.

Resembles *C. crassipalpis* and of about the same size. The mandibles of the male are similarly shaped, but the convexity and the internal ridge are less prominent. The male palpi are a little more slender and the patella proportionally longer. The process of the tibia has the upper tooth nearer the hook than in *crassipalpis*, making the process appear wider and stouter, fig. 2. The tarsus is a little smaller than in *crassipalpis*. Plate v, figs. 2*a*, 2*b*.

Salem and Marblehead, Mass.

**Clubiona tibialis**, new sp.

PLATE V, FIGURES 3, 3*a*, 3*b*.

Male 6·5$^{mm}$ long, another 5$^{mm}$ long. The male mandibles are slender and tapering, without any distinct ridges on the front. The male palpi are short and the tibia and tarsus both very large. The tibia is very complicated in shape, having a large hook on the outer side, a short thick process on the inner side, and a thickened edge in front that meets a slight elevation on the back of the tarsus. The tarsus is long and large, and so is the palpal organ. Plate v, figs. 3, 3*a*.

A female, apparently of this species, is $6^{mm}$ long. The front legs are shorter than in the male; the mandibles are stout and convex in front. The epigynum is large with a deep rounded notch in the middle and a slight ridge each side. Fig. 3*b*.

Eastern Massachusetts, and in N. Pike's Long Island collection.

**Clubiona canadensis**, new sp.

PLATE V, FIGURES 4, 4*a*, 4*b*, 4*c*.

Male $7^{mm}$ long, others smaller. Mandibles tapering and rounded, without ridges on the upper side. Male palpi short, tibia shorter than patella, with a complicated process on the outer side ending in a long sharp point with a round notch in the upper edge. Plate v, figs. 4, 4*a*. The tarsus is more than twice as long as wide, bent downward at the end. The palpal organ has a large bulb with small appendages at the end. Fig. 4*b*.

Female a little larger, epigynum with only two depressed spots just in front of the transverse fold. Fig. 4*c*.

Abdomen dark, with brown irregular lines. Cephalothorax pale, not darkened toward the front.

The common species on Mt. Washington, N. H., from the Glen to the highest trees, under stones and in moss; also from Montreal, Canada.

**Clubiona minuta**, new sp.

PLATE V, FIGURES 11, 11*a*, 11*b*.

This little spider is about $3^{mm}$ long and in its general appearance resembles a pale *C. rubra*. The male palpi, however, show it plainly to be a different species. The patella is longer than wide, as in *rubra*, and the tibia is short and wide at the end. Its appendage on the outer side is very simple, appearing from above like a thin spine at the side of the tarsus and not overlapping it. Pl. v, fig. 11. From the outer side it is seen to be flat, wide at the base, and tapering from the middle to a blunt point, fig. 11*a*. The palpal organ has a small hook on the inner side, fig. 11*b*, very different from the large hook of *C. rubra*.

Male from Readville, Mass., June 15, on bushes.

**Clubiona pusilla**, new sp.

PLATE V, FIGURES 5, 5*a*, 5*b*.

One male $6^{mm}$ long, another only $4^{mm}$. Head nearly as wide as the thorax.

Front row of eyes half as long as the head is wide, the eyes of equal size and equidistant; upper row, longer by the diameter of the lateral eyes. The upper eyes are larger than those of the front row, and all about the same size, the middle pair a little farther apart than they are from the lateral eyes.

Mandibles with a thin keel on the front outer edge, half as long as the mandible.

Colors, pale on the legs and palpi; cephalothorax brownish yellow, darkest in front; abdomen covered with fine brown markings.

Male palpi with the tibia shorter than the patella. A flat wide process on the outer side, Pl. v, fig. 5*a*, extends forward over the tarsus. The bulb of the palpal organ nearly fills the under side of the tarsus, the tube is short and curved round the end of the tarsus so as to point backward ; over the base of the tube is a short stout hook, instead of a large hook as in *rubra*, fig. 5.

Salem and Beverly, Mass.

**Clubiona rubra** Keyserling, specimens in Mus. Comp. Zool., Cambridge, Mass

PLATE V, FIGURES 6, 6*a*, 6*b*, 7, 7*a*, 7*b*, 8, 8*a*, 8*b*.

This is one of several closely similar species, the classification of which cannot well be understood without comparing large numbers from many different places. *Clubiona abbottii* L. Koch, is this species or very close to it.

Males and females are 3 to $4^{mm}$ long. The eyes are large in proportion to the size of the spider, and cover the whole width of the front of the head.

The color after keeping in alcohol is redder than in most species.

The epigynum is notched at the hinder edge, the depth of the notch varying in different individuals. Pl. v, figs. 6*c*, 7*c*.

The male palpi have the patella and tibia of the same length. The relative length of these joints differs in the allied species. The tibia is wider than long and has a large appendage on the outer side, divided from the main part of the tibia by a deep notch on the under side, figs. 6, 7, 8. The appendage consists of two parts, figs. 6*a*, 7*a*, 8*a*, the under one longest and a little notched at the end. The size and length of this process varies in different individuals. On the dorsal side of the tibia, on the front edge, is a small tooth, varying in size in different spiders, figs. 6, 7, 8. The palpal organ has a very large middle process, figs. 6*b*, 7*b*, 8*b*.

White Mountains, N. H., to Connecticut.

**Clubiona ornata,** new sp.

PLATE V, FIGURES 9, 9*a*.

Female $8^{mm}$ long. The abdomen is pointed behind and more narrowed in front than in most species. Both abdomen and cephalothorax are less flattened than in most species. The mandibles of the female are not very stout and less swelled at the base than usual.

This is one of the few species with a colored pattern on the abdomen. A dark stripe runs along the middle, of a different width in different individuals, but generally narrow and tapering behind. At the sides of this are two white or light yellow stripes with irregular edges, and beyond this the brown sides of the abdomen. Pl. v, fig. 9. The body is pale underneath. The epigynum is notched at the edge of the transverse fold, fig. 9*a*.

Mt. Washington, Dublin, N. H., and Massachusetts.

**Clubiona excepta** L. Koch.

*Clubiona pallens* Hentz.

PLATE V, FIGURES 10, 10*a*, 10*b*, 10*c*, 10*d*.

Female $7^{mm}$ long, cephalothorax $3^{mm}$. The abdomen is not usually much larger than the cephalothorax and unlike most species has a pattern on the back similar to that of *Amaurobius* and *Tegenaria*, or in very light individuals consisting of three rows of gray spots on a white or pale yellow ground. Pl. v, fig. 10*a*. The cephalothorax and legs are light yellowish brown, darkest on the head and mandibles. The spinnerets are rather long. The epigynum has two round brown spermathecæ that show through the skin, close together just in front of the transverse fold. In front of these are two oblique openings directed forward and inward.

The males are not much smaller than the females. The male palpi are slender, the tibia only a little longer than the patella, and the tarsus nearly as long as both together. The tarsus is oval, about half as wide as long, and rounded on the upper side. The papal organ is narrow and covered by the tarsus. On the inner side is a thin appendage, the free edge of which lies along the middle line and covers the long straight tube. On the outer side near the end of the tube is a straight process directed forward, and at its base a hook directed backward, fig. 10*c*. At the end of the tibia on the outer side is a short flat process with a small curved tooth on the upper corner, fig. 10*b*.

Massachusetts, Connecticut, and in N. Pike's Long Island collection. Under stones and leaves, sometimes in white cocoons.

## Chiracanthium C. Koch.

### Chiracanthium viride, new sp.

PLATE V, FIGURES 12, 12*a*, 12*b*, 12*d*.

Female 8$^{mm}$ long, cephalothorax 3$^{mm}$. Cephalothorax three-fourths as wide in front as at the widest part, fig. 12*a*. Eyes spreading over nearly the whole width of the head; the lateral eyes close together; the upper row a little longer than the front row, eyes in both rows at equal distances apart. Abdomen widest in the middle, tapering behind. First pair of legs a third longer than the fourth. Sternum widest just behind the first pair of legs and tapering to a point between the fourth coxae. The mandibles and maxillæ are dark brown. The rest of the body is pale yellow, the cephalothorax a little darker than the rest, and a gray stripe covers the middle of the front of the abdomen. The epigynum has a large oval opening covered by a hard dark brown lump. Pl. v, fig. 12*d*.

In the male the mandibles and legs are longer and the difference in length between the first and fourth legs is greater. The palpi are as long as the second femur. The tibia is twice as long as the patella, and has on the outer side a thin black process, a little curved toward the tarsus, and on the upper side a thick blunt process extending a little way over the back of the tarsus. Between the two processes of the tibia a sharp process of the tarsus extends backward, a little curved down at the end, figs. 12*a*, 12*b*.

Female, Dedham, Mass., July 26. Male, Saugus, Mass., June 12.

## Trachelas L. Koch.

### Trachelas ruber Keyserling.

PLATE V, FIGURES 13, 13*a*, 13*c*, 13*d*.

Female 10$^{mm}$ long, cephalothorax 4$^{mm}$ long and 3$^{mm}$ wide. The cephalothorax is widest in the middle opposite the second pair of legs and narrows to 2$^{mm}$ at the hinder end, the sides of the hinder half being nearly straight. The head is very wide and high, the highest part half way between the eyes and the dorsal groove.

The eyes are all about the same size and far apart. The front row is nearly straight, the middle eyes a little higher than the lateral, this row is half as long as the head is wide. The hinder row is much longer, the middle eyes are about as far from the front middle pair as they are from each other, the lateral eyes are about the same distance from the middle ones, but much farther back on the head, figure 13. The mandibles and maxillæ are large and resem-

ble those of *Clubiona*. The abdomen is oval and very regular in shape. Pl. v, fig. 13. The cephalothorax is very thick and hard, and dark brown. The abdomen is light yellow with no markings, except four small brown spots near the middle, and a gray streak over the dorsal vessel. The hairs are very short and scattered so that the skin appears soft and smooth.

The *first pair of legs* is a little the longest instead of the fourth pair, as in the European species, and both the first and second pairs are much stouter than the third and fourth. The palpi are slender, the tarsal joint thickened at the tip. The legs are darker from back to front, the front pair reddish brown, not so dark as the cephalothorax, and the hind pair is yellow. The epigynum has two dark brown round depressions close together.

Pale individuals are sometimes found with all the legs yellowish white, cephalothorax light brown with white eyes, and the abdomen light gray.

The males are smaller than the females, sometimes not more than half as large. The tibia of the palpus is shorter than the patella, and has a short hook on the outer side. The tarsus is small and the bulb of the palpal organ is so large that it extends beyond the tarsus on both sides. The bulb is round and has a distinct tube which rests in a groove of the end of the tarsus, figs. 13*c*, 13*d*.

Under stones and leaves and sometimes on fences in autumn. In general appearance and color it resembles *Dysdera*. Massachusetts and Connecticut, and in N. Pike's Long Island collection.

## Ahyphæna Sundevall.

PLATE VI, FIGURES 1, 1*a*.

Cephalothorax highest behind. Eyes of the front row equal in size and equidistant, the lateral eyes a little the highest. Upper row of eyes longer than the front row, the middle eyes highest, all of the same size and larger than those of the front row and at equal distances apart. Abdomen widest in the middle and a little pointed behind.

Maxillæ long and widened at the tips but not so much widened as in *Clubiona*.

The opening of the tracheæ is farther forward than in other genera, in some species approaching nearly to the epigynum. Pl. VI, fig. 1*a*.

The colors are pale. The male palpi are large and complicated.

**Anyphæna rubra**, new sp.

PLATE VI, FIGURES 1, 1*a*, 1*b*.

Female 8 or 9$^{mm}$ long, cephalothorax 3$^{mm}$. Abdomen half longer than the cephalothorax and about as wide, tapering backward from the middle to the spinnerets, Pl. VI, fig. 1. The cephalothorax and legs are pale yellowish brown. The cephalothorax has two darker longitudinal bands. The abdomen is white or light yellow with two stripes made up of brown or red spots. The mandibles are dark brown. This is the largest and stoutest species.

The epigynum has two large curved openings, turned toward each other, between which is a long depression widened at the front end. The long spermathecæ show through the skin just behind the openings, fig. 1*b*. I have not seen the adult male.

Massachusetts and Connecticut, and in N. Pike's Long Island collection. On plants and under stones.

**Anyphæna incerta** Keys., specimens in Mus. Comp. Zool., Cambridge, Mass.

PLATE VI, FIGURES 2, 2*a*, 2*b*, 2*c*, 2*d*.

Female 5$^{mm}$ long, cephalothorax 2$^{mm}$. The cephalothorax is about a quarter longer than wide, rounded at the sides, and highest in the middle. The front of the head is very low, so that the front eyes are not their diameter from the base of the mandibles. The front row of eyes is nearly straight. The upper row is longer and more curved, with the middle eyes highest, and the eyes of this row are all larger than those of the front row. The abdomen is large in the female, as in all the species of this genus, widest just behind the middle and a little pointed behind, Pl. VI, fig. 2.

The color is light brownish yellow with gray markings. The cephalothorax has two indistinct longitudinal stripes and a fine black line over the legs on each side. On the abdomen are two rows of faint spots and oblique lines. The legs have a few faint markings across the joints.

The maxillæ are straight at the sides and rounded at the ends on the inner side. The labium is small and not half as long as the maxillæ.

The epigynum has a large dark brown process in the middle at the front end, fig. 2*d*.

The tibia of the male palpus has a large double process on the outer side, the upper branch of which is pointed, and the lower blunt with a rounded tooth on the upper side, figs. 2*a*, 2*b*, 2*c*.

Under leaves in winter, Salem and Swampscott, Mass.

**Anyphæna calcarata,** new sp.

PLATE VI, FIGURES 3, 3*a*, 3*b*, 3*c*, 3*d*.

The same size as *A. incerta*, but lighter colored and with longer legs and longer spines. The front legs are longer than the fourth in both sexes. The markings are the same as in the other species and the spots on the front of the abdomen are more distinct than in the others.

The epigynum has a thin edge extending backward a little over the transverse fold and reaching from one respiratory opening to the other. In the middle is a small hole with a short tooth-like ridge directed backward on each side. Pl. VI, fig. 3*d*.

The male palpi have the outer half of the femur twice as thick as the base with a few large spines on the upper side near the end. The patella is as wide as long and shorter than the tibia. The appendage on the outer side of the tibia is very small and does not extend forward beyond the base of the tarsus, fig. 3*b*, 3*c*; near the base of the tibia on the under side is a blunt tooth, fig. 3*a*, 3*c*. The tarsus resembles that of *A. incerta*. The palpal organ has the middle process very stout and curved inward at the end, fig. 3*a*.

The coxæ of the fourth pair of legs have on the under side a small pointed process directed outward. The coxæ of the third pair have on the under side a curved process directed inward with a short tooth on the binder side near the middle, and in front of this a short blunt tooth directed backward, fig. 3.

West Haven, Conn., July, on plants, and in N. Pike's Long Island collection.

**Anyphæna saltabunda.**

*Clubiona saltabunda* Hentz.

PLATE VI, FIGURES 4, 4*a*, 4*b*, 4*c*, 4*d*.

This is a very long-legged and slender species. The female is $4^{mm}$ long, the abdomen but little longer than the cephalothorax. The front leg is $10^{mm}$ long, fourth leg $7^{mm}$. The palpi are slender and as long as the femora of the first legs. The whole body is white with two broken gray bands on the cephalothorax and two rows of gray spots on the abdomen.

The male is about as large as the female. The male palpi are long, the tibia of very complicated shape. It is curved outward and has near the base on the outer side a long, thin forked process. Pl. VI, fig. 4. The tarsus is of the usual shape. The palpal organ has a

short slender tube resting against the tip of the tarsus. Behind the tube is a thin hooked process, and on the inner side a long process with small black teeth at the end, fig. 4, 4*a*, 4*b*, 4*c*.

The epigynum has a long transverse opening a little in front of the fold, fig. 4*d*.

Massachusetts, and Meriden, Conn.

## Phrurolithus Koch and Westring.

*Micariosoma* Simon.

Small spiders sometimes with bright markings and iridescent scales. The legs of the first and second pairs have a double row of strong spines under the tibia and metatarsus. Pl. VI, fig. 5*b*. The maxillæ are short and wide. The palpi of the males are very large compared with the size of the spider, and have a long stout process on the outer side of the tibia. The arrangement of the eyes and the pattern of the dorsal markings resemble those of *Agroeca*.

## Phrurolithus pugnatus, new sp.

PLATE VI, FIGURES 6, 6*a*, 6*b*, 6*c*.

$2^{mm}$ to $3^{mm}$ long. Cephalothorax round, narrowed at the head as in *alarius*. Abdomen usually shorter and rounder than in *alarius*. Pl. VI, fig. 6. Cephalothorax and legs bright yellowish brown. Abdomen dark brown with transverse light markings which vary in different individuals. Light yellowish beneath, except around the spinnerets and epigynum.

Epigynum with two oblique openings at the front end farthest from the transverse fold. Parts of the palpal organ are sometimes found in the openings of the epigynum, fig. 6*e*.

The male palpi are large in proportion to the size of the spider. The femur has a short process near the base on the inner side. The patella is as short as wide, but the tibia is nearly as large as the tarsus and wider at the distal end. On its inner side is a long stout tooth projecting forward, and on the outer side a longer curved one as in *P. alarius*. The tibia is oval and the palpal organ short and round, not extending backward at the base as in *alarius*, figs. 6*a*, 6*b*.

*Herpyllus parcus* Hentz resembles this species.

Massachusetts and Connecticut.

## Phrurolithus alarius.

*Herpyllus alarius* Hentz.

PLATE VI, FIGURES 5, 5*a*, 5*b*, 5*d*, 5*f*, 5*g*, 5*h*.

Full grown female $4^{mm}$ long, cephalothorax $1.5^{mm}$. Pl. VI, fig. 5.

The cephalothorax is nearly as wide as long, rounded at the sides. The head is about half as wide as the thorax and the eyes are close together and all about the same distance apart, fig. 5*a*. The middle eyes of the upper row are oval and turned obliquely, nearest together towards the front.

The abdomen is oval, widest behind, and a little flattened on top. The legs are long and slender, except the tibiæ and metatarsi of the first and second pairs which are twice as thick as the same joints of the other legs, fig. 5. The legs are light yellow or white with gray hairs, except the tibia and patella of the first pair, which are black or dark gray with the tip of the tibia white. The tibia and patella of the second pair are marked with lighter gray in the same way. The tibia and metatarsus of the first and second pairs have two rows of strong black spines on the under side, fig. 5*b*.

The cephalothorax is light yellowish with a black edge each side and a few irregular radiating gray marks forming two indistinct longitudinal stripes. The abdomen is gray with transverse white markings which vary greatly in shape and size in different individuals, figs. 5, 5*a*. The abdomen is covered with flat branched hairs that are iridescent, changing from light grayish-green to pink with the motions of the spider, fig. 5*d*. The under side of the body is pale with a dark mark in front of the spinnerets, and in some individuals a few irregular marks along the sides.

The male palpi are large. The femur is thickened on the under side near the outer end, forming a short black process covered with short stiff hairs. The patella and tibia are both short. The tibia has on the outer side a long process slightly curved downward that extends along the side of the tarsus for half its length, fig. 5*g*. The palpal organ is so long that its base extends over the end of the tarsus, fig. 5*f*.

The epigynum has two large openings turned toward the sides a little in front of the transverse fold, fig. 5*h*.

It lives on and under stones in dry open ground and runs with great swiftness short distances at a time. When still it lies close to the stone with the tibiæ drawn up over the back, as in fig. 5, the thickened and colored legs of the first pair are then the parts of the spider most easily seen.

Massachusetts and Connecticut.

### Agrœca Westring.

**Agrœca pratensis,** new sp.

PLATE VI, FIGURES 7, 7*a*, 7*b*, 7*c*, 7*d*, 7*e*.

Female $7^{mm}$ long, cephalothorax $3^{mm}$. The cephalothorax is widest and highest behind the middle, the head a little more than half as wide as the thorax. The abdomen is widest across the hinder third and not much pointed behind. Pl. VI, fig 7. The front row of eyes are close together, the middle ones half their diameter highest. The upper eyes are a little larger and about their diameter apart, the middle pair much the highest, fig. 7.

The mandibles are very convex in front and flat at the sides. The maxillæ are straight on both sides and a little rounded on the inner corners. The labium is half as long as the maxillæ and as wide as long. The sternum is large and as wide as long, fig. 7*a*. The legs are stout, the fourth pair longest. Under each metatarsus are three pairs of slender spines, under the first and second tibiæ two pairs, and under the third and fourth tibiæ three pairs. The cephalothorax, legs, and mouth parts are light brownish yellow. The cephalothorax has a fine dark edge on each side and a row of radiating dark lines each side forming two broken dark longitudinal bands. The abdomen has two rows of gray oblique markings on a light ground, fig 7. Epigynum with a long brown piece in the middle, fig 7*e*.

Male about the same size with the abdomen a little smaller. The male palpi are large and stout. The patella and tibiæ are the same length, the latter a little bent and with a short pointed spine on the outer side. The tarsus and palpal organ are short and wide. The palpal organ has a short blunt process on the outer side that projects over the edge of the tarsus, figs. 7*b*, 7*c*, 7*d*.

This spider lives under leaves and in short grass and resembles a *Lycosa* in its gait and general appearance, and also the common *Anyphæna incerta.*

Eastern Massachusetts; Providence, Rhode I.; Albany, New York.

## Agalenidæ.

The *Agalenidæ* have the cephalothorax longer than wide, with the cephalic part higher than the thoracic, and distinctly separated from it by grooves or marks at the sides. The head is usually higher than in the *Drassidæ* and the body less flattened. The upper spinnerets are two jointed, the terminal joint pointed and provided with spinning tubes along the inner side. In most species these are longer

than the other spinnerets. The feet have three claws. The *Agalenidæ* make large flat or irregular webs with a tube or hiding place at one side from which they run out and seize the insects that alight on the web. The *Agalenidæ* run on the upper side of the web with their back upward, while *Linyphia*, which makes similar flat webs, runs on the under surface, back downwards.

## Cœlotes Blackwall.

The difference between *Cœlotes* and *Tegenaria* is not a very distinct one. I have placed in Cœlotes those stouter and shorter legged species with the mandibles prominent in front, and in which the palpi of the males have processes on both patella and tibia.

The eyes are in two rows, nearly straight, and differ but little in size and distance apart. The mandibles are stout and convex. The maxillæ are wide at the ends, rounded on the outer corners, and obliquely truncated on the inner. The labium is about half as long as the maxillæ, a little narrowed and truncated at the tip. The colors are dark gray and brown.

### Cœlotes medicinalis.

*Tegenaria medicinalis* Hentz.

PLATE VII, FIGURES 1*a*, 1*b*.

Female $12^{mm}$ long; cephalothorax $5^{mm}$, fourth leg $15^{mm}$. Front row of eyes straight, the middle eyes largest, eyes of upper row all of the same size and about equal distances apart, the middle eyes highest. The lateral eyes of both rows are close together, those of the upper row farthest toward the sides. Head high and wide, distinctly separated from the thorax by grooves each side. Abdomen oval, widest behind. Legs moderately stout.

Cephalothorax yellowish brown, darkest in front, marked with radiating gray lines forming two longitudinal dark bands. Abdomen gray with irregular pale spots. A double row of oblique pale spots in the middle, in most specimens broken and irregular, fig. 1. The legs are light yellowish brown with light indistinct gray rings.

The spinnerets are short.

The epigynum has a large piece in the middle with a branch each side that extends outward and forward ending in a point. In front are two small rounded teeth directed inward. Pl. VII, fig. 1*a*.

Male palpus short, with a large and wide tarsus. The patella and tibia are both short and of about the same length. The outer pro-

cess of the patella is half as long as the patella and blunt and crooked at the end. The tibia has on the upper side a short pointed process near the base and a blunt one near the end on the outer side, both concealed by a curved ridge. On the under side of the tibia is a process directed forward. The tube of the palpal organ is slender and supported at the end by two large processes, fig. 1*b*.

Chateaugay Lake, Adirondacks, N. Y., from F. A. Bowditch, 1878; Swampscott, Mass., May 8.

## Cœlotes longitarsus, new sp.

PLATE VII, FIGURES 2, 2*a*.

Male $7^{mm}$; cephalothorax $3{\cdot}5^{mm}$. Head wide and high, highest half way between the eyes and the dorsal groove. First leg almost as long as the fourth. Legs stout. Abdomen oval, the hinder half a little the wider. Cephalothorax and legs yellowish brown, a few radiating darker lines on the thorax, and the front of the head a little darker. Legs darker toward the tips. Abdomen dark gray with a median lighter stripe in front and a double row of lighter oblique marks on the hinder half, much as in *medicinalis*.

The patella and tibia of the male palpi are both as short as wide. The patella has a long tooth, widest and truncated at the tip, directed forward on the outer side. The tarsus is widest at the base and pointed at the tip. At the base on the outer side it has a stout process extending backward and inward as far as the patella. Plate VII, fig. 2.

A small female found at the same time and probably the same species has a small, simple oval opening at the posterior part of the epigynum. Fig. 2*a*.

Mt. Carmel, Conn.

## Cœlotes montanus, new sp.

PLATE VII, FIGURES 3, 3*a*.

$12^{mm}$ long; cephalothorax $5^{mm}$; 4th leg of female $18^{mm}$, 4th leg of male $20^{mm}$.

Epigynum with the holes open, and oblique turned nearly forward. Plate VII, fig. 3.

Male palpus with short patella and tibia, the tibia shortest. The patella has a short conical, black spine on the outer side near the end, directed forward, under this is a smaller spine. On the outer side of the tibia, which is shorter than the inner side, is a short process turned forward a little at the tip. Fig. 3*a*.

The colors and markings are like those of the two preceding species. The legs, especially of the male, are a little longer in proportion to the size of the body.

Chateaugay Lake, Adirondacks, N. Y., from F. A. Bowditch. A small male from New Haven, Conn., is apparently of this species. Its palpi differ slightly from those of the Adirondack males as shown in figs. 4, 4*a*.

### Cœlotes hybridus, new sp.

PLATE VII, FIGURES 4, 4*a*.

This species is only distinguished from *C. longitarsus* by small differences in the shape of the male palpi. The spur at the base of the tarsus is very short and extends backward to a short process on the middle of the tibia. The posterior inner corner of the tarsus is differently shaped from this part in *longitarsus*, having a deep notch shown in figs. 4, 4*a*. The tibia is a little longer than that of *longitarsus* and shaped much like that of *medicinalis*. The patella resembles that of *longitarsus*. In size, markings, and colors this is like both *medicinalis* and *longitarsus*. The palpal organ is almost exactly like that of *longitarsus*.

One male from Chateaugay Lake, Adirondacks, N. Y., from F. A. Bowditch.

## Tegenaria Latr.

These spiders differ from *Cœlotes* in having the legs longer and more slender and the abdomen generally rounder and shorter. The mandibles are less convex in front, the palpi of the males have no processes on the patella, and the palpal organ is proportionally smaller than in *Cœlotes*.

### Tegenaria derhamii Scopoli, 1763; Thorell, 1873.

*Tegenaria civilis* Blackwall, 1861.
*Tegenaria domestica* Simon, 1875.

PLATE VII, FIGURES 6, 6*a*, 6*b*, 6*c*.

This is a house spider found all over the world. Female $10^{mm}$ long; cephalothorax $5^{mm}$. Plate VII, fig. 6. The legs are long and tapering, 4th leg $18^{mm}$ long. Tarsi and metatarsi slender. The head is high and wider in front than it is opposite the first pair of legs. The abdomen is short, only a little longer than the cephalothorax. The front row of eyes is straight, the middle ones smallest. Upper row longer, lateral eyes close to those of the front row, the middle ones much higher, fig. 6.

Cephalothorax and legs light yellowish brown, the legs with some indistinct gray rings. Abdomen pale with gray markings which are usually plainest on the hinder half. The upper spinnerets are twice as long as the lower and the terminal joint is nearly as long as the basal.

Epigynum with small oblique openings at the sides. Fig. 6*c*.

The male palpi have the patella and tibia long and both about the same length. The tibia has on the outer side, about one-fourth its length from the end, a short blunt process, fig. 6*a*, under this is a shorter process lighter colored and directed forward, fig. 6*b*. The tarsus is slender and pointed and the appendages of the palpal organ are small. Figs. 6*a*, 6*b*.

### Tegenaria brevis, new sp.

PLATE VII, FIGURES 5, 5*a*, 5*b*, 5*c*.

A small species, 5 to 6$^{mm}$ long. Cephalothorax two-thirds as wide as long. Abdomen short and widest behind. The mandibles are small and but little convex in front. The cephalothorax and legs are pale yellowish brown with black hairs, the legs and palpi are lightest at the base and darker toward the ends. The spines are very long and slender. The abdomen is in some individuals pale with dark hairs; in others there is a gray herring-bone marking, and gray marks along the sides.

The male palpi are slender and without any appendages on the patella. The tibia has a short stout tooth on the outer side a little behind the end. The tarsus is small and pointed. The palpal organ is round and too large to be covered by the tarsus. Plate VII, fig. 5. The head of the male is narrower than that of the female, and the thorax wider, figs. 5*b*, 5*c*.

The epigynum appears to the naked eye like two parallel dark brown marks. It has a large posterior opening, widest behind, and partly divided into two at the front edge, fig. 5*a*.

The short round abdomen and gray markings make this spider resemble *Steatoda marmorata*.

Mt. Washington. N. H.; Massachusetts; New Haven, Conn.

### Cicurina Menge, 1871. Simon, 1875.

This genus differs but little from *Cœlotes* except in the palpi of the males which have the patella without processes, the tarsus long and narrow, and the tibia short with a large appendage on the outer side. The tube of the palpal organ is long and supported in various complicated ways.

**Cicurina complicata**, new sp.

PLATE VII, FIGURES 7, 7*a*, 7*b*.

This is a small, stout species, the largest individuals measuring $7^{mm}$ in length, and the cephalothorax $3^{mm}$. The cephalothorax, legs, and palpi are uniform yellowish brown. The abdomen is pale with scattered gray markings both above and below, in some individuals forming an indistinct herring-bone pattern on the dorsal side. The sternum is as wide as long and nearly as wide in front as in the middle. The mandibles are stout, and in the females very convex in front. In the males the head is narrower and the mandibles smaller.

The palpi of the males have the patella simple and about as long as wide. The tibia has a short tooth near the base on the outer side, the middle part is turned inward, and on the outer side at the end is a large flat and crooked appendage that in its natural position appears to be part of the palpal organ. Plate VII, fig. 7. In fig. 7*a* the process is shown from the side partly turned away from the palpal organ. The tarsus is long and narrow and rounded at the tip, fig. 7*b*. The tube of the palpal organ is very long, beginning at the base of the tarsus it runs along the inner side around the tip, where it is supported by the edge of a flattened appendage, and backward along the outer side, the end being under the flat tibial process.

The epigynum has a simple oval opening behind, and the tubes show through the skin in light-colored individuals.

Blue hill, Milton, Mass., and Salem, Mass., under leaves, in winter.

## Hahnia Koch.

Upper eyes all about the same size, the middle pair highest and farthest back and farther from each other than they are from the lateral eyes. Front eyes nearer together, the middle ones highest. The lateral eyes of both rows close together.

The spinnerets are in a single transverse row, the upper pair being outside the others. The outer pair has two nearly equal joints and the next pair have a short second joint at the tip.

The opening of the tracheæ is in the middle of the abdomen instead of directly in front of the spinnerets, as in most of the family.

Small spiders resembling *Tegenaria* and *Cœlotes*, except in the spinnerets.

## Hahnia bimaculata, new sp.

PLATE VII, FIGURES 8, 8*a*, to 8*f*.

Length, 2·5$^{mm}$. Eyes large, both rows with the lateral eyes lowest. Lateral eyes much nearer together than the middle pair. Front eyes a little the larger, both rows strongly curved, the middle eyes highest. Maxillæ short and wide, the front edge straight except on the inner corner. Sternum as wide as long, widest opposite the second legs. The spinnerets are long, the terminal joints of the outer pair nearly as long as the basal joint. The tracheal opening is nearer the epigynum than the spinnerets. Plate VII, fig. 8.

The cephalothorax, sternum, and mouth parts are reddish brown. The abdomen is light gray with many irregular pale spots and a double row of pale oblique markings in the middle. In the middle of the front half of the abdomen are two orange colored spots. The under side of the abdomen is pale with a few gray spots. The legs are pale with gray rings, two rings on the femur, tibia, and metatarsus. The skin over the epigynum is very transparent and shows two convoluted tubes almost always unsymmetrical, figs. 8*c*, *d*, *e*, *f*. The male palpus has on the outside of the tibia, near the end, a pointed process as long as the diameter of the tibia. At the base of the patella on the outer side is a small pointed black process curved a little forward, fig. 8*b*. The tarsus is nearly as wide as long but slightly pointed at the tip. The palpal organ is flat with a long thin tube extending along the inner side around the end, fig. 8*a*.

Common under dead leaves. Massachusetts, Connecticut, and Mt. Washington, N. H.

## Hahnia radula, new sp.

This spider resembles *H. bimaculata* but is nearly twice as large. On the under side of the first and second legs and palpi of the male the hairs are raised on short transverse ridges so that the leg appears serrated when seen from the side. Plate VII, figs. 10, 10*a*. The gray markings of the abdomen and rings around the legs are less distinct than in *bimaculata*. The little spine at the base of the patella of the male palpi is shorter than in *bimaculata* and sharply turned forward. The appendage of the tibia is the same as in *bimaculata*, and the tarsi and palpal organs are very similar.

One male, Jaffrey, N. H., Aug. 1.

**Hahnia cinerea,** new sp.

PLATE VII, FIGURES 9, 9*a*, 9*b*.

Length, 1·5 to 2$^{mm}$.

Cephalothorax light, with dark radiating markings. Abdomen dark gray with scattered small white spots and a double median row of oblique light markings somewhat like *Cœlotes*. Plate VII, fig. 9. The legs are light yellowish brown with patellæ, coxæ, and the ends of the longer joints paler than the rest. The basal joints of the spinnerets are light yellowish brown like the legs. Terminal joint of outer spinnerets shorter than basal. The tracheal openings are nearer the spinnerets than the epigynum. The skin over the epigynum is rather opaque and but little of it can be seen. The palpi of the male have the patella and tibia both short and each has a long, slender process on the outer side which is flexible and variously curved at the end, fig. 9*b*. The tarsus is short and oval. The palpal organ has at the base a short feather-like appendage. The tube is slender and curved around the distal end of the tarsus. It has near the end a short soft appendage of the bulb, fig. 9*a*.

Salem, Beverly, Swampscott, Cambridge, Roxbury, Mt. Tom, Mass., and New Haven, Conn.

## Agalena Walck.

Large hairy spiders with long legs and very long upper spinnerets. The head is high and the middle eyes of both rows are much higher than the others. The web is flat and more regular and closely woven than in *Tegenaria*.

**Agalena nævia** Walck. and Bosc., 1841; Hentz, 1848.

*Agalena potteri* Blackwall, Ann. and Mag. Nat. Hist., vol. xvii, 1846.

*Agalenopsis albipilis* Giebel, Zeitsch. Gesammt. Nat., 1869.

*Agalena americana* Keys., Zool. botan. Gesellsch., Wien, 1877, male with short-tubed palpal organs, from Illinois.

PLATE VIII, FIGURES 1, 1*a*, 1*c*, 1*d*, 1*f*, 1*g*, 1*h*, 1*i*, 1*j*, 1*k*, 1*l*, 1*m*, 1*n*.

This is the common grass spider all over the United States. It varies greatly in size. A large male measures 14$^{mm}$ long, 4th leg 35$^{mm}$. A large female, 18$^{mm}$ long, 4th leg 30$^{mm}$, while a small adult male is only 7$^{mm}$ long, and the 4th legs 15$^{mm}$. Plate VIII, fig. 1.

The cephalothorax is long and the cephalic part separated distinctly from the thoracic by grooves radiating from the dorsal depression. The head is high and wide in front and contracted a little just in front of the first pair of legs. The two rows of eyes

are strongly curved, the lateral much lower than the middle pairs, so that the middle front eyes and the lateral hinder eyes form together a nearly horizontal line. The thorax is marked by radiating grooves between the legs. The abdomen is about twice as long as wide, widest in the front half, a little truncated in front where it overlaps the thorax, and tapering behind. The upper spinnerets are two or three times as long as the under. The legs are long and tapering, the fourth pair longest. The maxillæ are much widened at the tips and nearly straight on the front edges.

The cephalothorax has two wide longitudinal dark stripes. The abdomen has a light longitudinal stripe in the middle, straight in front, and herringbone-shaped in the hinder half, generally a little darkened in the middle and lightest at the edges. The sides of the abdomen are dark, or covered with dark spots close together toward the middle stripe and more scattered toward the sides. On the ventral side the abdomen has a middle dark stripe, sometimes lighter in the middle.

Though the markings vary but little the colors vary from light yellow, with pale gray markings, to dark reddish brown, with black and gray spots, the colors being usually modified by long gray hairs both in dark and light individuals. The joints of the legs are all dark toward the end. Large individuals are, as a rule, darker colored than small ones.

The palpi are long in both sexes. In the males the femur is long, the patella not much longer than wide, and without appendages: the tibia about as long as patella, widened at the distal end, the outer side extending forward along the edge of the tarsus and having a short blunt tooth, figs. 1*c*, 1*g*, 1*d*. The tarsus is large, the basal half oval and the tip narrowed into a long point. The palpal organ, especially the tube, which is largely developed in this species, instead of having a constant form, as in most spiders, varies extremely. The most common form is that shown in figs. 1*a*, 1*g*, with a stout flat tube coiled in one and a half turns under the tarsus, and with the tip turned outward away from the tarsus. On the outer side of the palpal organ near the end of the tube is a short thin tooth with the outer edge turned downward and the corner usually forming a blunt tooth directed toward the end of the palpus. This variety is found in spiders of all sizes and shades of color from all parts of the country. Among large spiders from various localities occurs the form of palpal organ shown in fig. 1*b*. In this the tube is much longer and more slender, and terminates in a sharp

point turned inward toward the tarsus. A third variety, shown in fig. 1*d*, occurs less often but on spiders of all sizes and from different parts of the country. The spiral here hardly makes more than one turn and is so small as to be covered entirely by the tarsus. At the tip the tube is twisted so as to turn the opening downward. This is the form named *Agalena americana* by Keyserling, in 1877, from Illinois; there is one in the Cambridge museum, from Penikese Island, Mass., named by Keyserling "var. *americana*," and I have seen specimens from Indiana, from Providence, R. I., New Bedford, Mass., and Brooklyn, N. Y., in N. Pike's collection. These three varieties seem to be distinct and I have seen no intermediate forms. Fig. 1*e* is a palpal organ from Providence, R. I., having an unusually large tube; fig. 1*f* is the palpus of a small spider from Jaffrey, N. H., in which the tube is slender and the spiral unusually small.

The shape of the external opening of the epigynum is even more variable than that of the palpal organ. The most common variety is shown in fig. 1*h*, taken from a female found in copulation with the male from which the palpus fig. 1*a* was drawn. Figs. 1*i*, 1*j*, 1*k*, show a slight variation from this form by short teeth on the front edge of the opening. Figs. 1*l*, 1*m*, 1*n*, have these teeth united and extending backward across the opening nearly dividing it into two. The three last are all from large dark colored spiders like those having palpi as in fig. 1*b*.

Comparison of a large number of specimens from the neighborhood of Boston, Mass., showed that 69 males had palpi like fig. 1*a*, and 5 like fig. 1*b*; 98 females had the oval epigynum, fig. 1*h*, and 37 the partly divided epigynum, figs. 1*l*, 1*m*, 1*n*.

The web of this species consists of a flat sheet, shaped according to the supports to which it is fastened, from one side of which extends a tube at the mouth of which the spider usually stands. The tube is open at the lower end, from which the spider escapes if the web is entered by too large an enemy.

The webs are made in all kinds of places. In early summer great numbers are made on short grass, but large webs are seldom made in such situations and it is probable that spiders that do not find more favorable places as they grow larger, never live to become adult. The largest webs and the best developed spiders are found among stones and shrubs where there are convenient hiding places and supports for the web, which in a good situation is enlarged as the spider grows until it becomes a foot or more wide and proportionally thick and strong. The long spinnerets are used in making

this flat web, the spider walking along slowly, swinging the spinnerets from side to side, making a band of very fine threads at each stroke. The web does not appear to be at all adhesive, it merely offers insects a convenient place to rest upon and the spider depends on his quickness of movement for their capture. Large webs usually have many supporting threads running up into fences and bushes and these perhaps help to trip the wings of flying insects and cause them to fall on the web, as similar threads do in the webs of Linyphia.

The pairing of this spider takes place on the web of the female. The female lies still with feet drawn up as if dead. The male lays her on one side under his thorax with her ventral side forward and inserts one of his palpi into the epigynum at frequent intervals for a long time, the soft parts of the palpal organ suddenly swelling and again contracting. When tired with one palpus he turns the female around and over so that she lies on the other side with her head in the opposite direction and uses the other palpus. The eggs are laid in a flat white cocoon, usually covered with a thick flat cone of silk with which considerable dirt is often mixed. The eggs are laid under stones or bark and on fences and buildings of all kinds, where they are partly sheltered, from August to October, and the females often remain and die on or near the cocoon. Adults are occasionally found under leaves in winter, but it is doubtful if any live until the next season. The eggs hatch early in the spring and the young spiders come out in May.

It appears to be the most common spider all over the United States.

## Dysderidæ.

Spiders with only six eyes and with the openings of the tracheæ in the front of the abdomen, just behind those of the air sacs, so that they appear to have four air sacs like the *Mygalidæ*. The family is a small one and the genera differ greatly in the structure of the feet and mouth parts.

### Dysdera interrita Hentz.

PLATE VIII, FIGURES 2, 2*a*, 2*b*, 2*c*, 2*d*.

Female 12$^{mm}$ long. Cephalothorax 5$^{mm}$ long and 3$^{mm}$ wide. The front of the head is wide and curved forward in the middle. The eyes are small and close together. Pl. VIII, fig. 2. The mandibles are half as long as the cephalothorax and inclined forward and much narrowed toward the end. The maxillæ are small, pointed at the

tips and widest half way to the base of the palpus. Fig. 2*a*. The labium is long and widened at the base. The sternum is widest in the middle, narrowed behind, and truncated at the front end. The coxæ are very long, fig. 2*a*. The first legs are longest and the fourth next. The feet have only two claws and under them a thick brush of flattened hairs. Fig. 2*b*. The patellæ are only about a fourth shorter than the tibiæ. The abdomen is long, oval and a little pointed behind. The cephalothorax and mandibles are reddish-brown. The legs are lighter colored and more yellow and become a little darker from behind forward. The abdomen is dirty white or yellow without markings.

The male differs little from the female. The palpal organ is as long as patella and tibia of the palpus. The terminal half is a little curved inward, and on the outer side is a short blunt tooth a little curved upward. Figs. 2*c*, 2*d*.

Swampscott, Brookline, and Roxbury, Massachusetts. This is the only *Dysdera* I have seen from New England, and as Hentz's *D. interrita* came from Massachusetts, this is probably the species. It agrees very closely with *D. crocata* Koch = *D. rubicunda* Blk. The palpal organ of *D. interrita* is straighter, as seen from in front, than that of *crocata*.

## Ariadne Savigny and Audouin.

### Ariadne bicolor.

*Pylarus bicolor* Hentz.

PLATE VIII, FIGURE 3, 3*a*, 3*b*, 3*c*, 3*d*.

Female 9$^{mm}$ to 10$^{mm}$ long. Plate VIII, fig. 3. Cephalothorax long and narrow, widest opposite the third pair of legs. In the male the cephalothorax is proportionally much wider. The dorsal groove is very small, and the head is not separated very distinctly from the thorax. The abdomen is oval, widest across the middle. The first, second, and third pairs of legs are turned forward. The first pair is longest, the second next. The legs are all stout and the first and fourth pairs have the patella and tibia much thickened. The color of the cephalothorax and legs is darker from behind forward, the fourth legs being light yellow and the first legs and front of the head dark brown. The abdomen is pale at the sides and dark purplish-brown above and below, darkest along the middle of the back.

The feet have three claws, fig. 3*d*. The tibia and metatarsus of the first and second legs have two rows of strong spines on the under side, four pairs on the tibia and eight or ten pairs on the metatarsus. The sternum is long and widest in the hinder half. The maxillæ are

long and narrow, widened a little half way btween the tip and the insertion of the palpus. The palpi are short and stout, fig. 3. The middle eyes are close together. The upper lateral eyes are about twice their diameter from the middle pair, and the front eyes are close to them, about half their diameter nearer the middle line.

The male is a little smaller than the female and has the thorax wider and the legs longer and more slender, fig. 3*a*. The metatarsus of the first feet is crooked at the base with a spine on each side, the outer one nearest the base, fig. 3*a*. The male palpi are but little longer or stouter than those of the female. The tibia is a little thickened. The palpal organ is attached to the under side of the tarsus; it has a round bulb about as thick as the tibia is long, which narrows on the outer side into a short finely pointed tube that curves sharply inward, fig. 3*a*.

It lives under stones and leaves, or in long yellowish tubes only wide enough to hold the spider under stones or in cracks of trees. In July and August the cocoon with twenty or thirty eggs is made in the tube with the female, and the young come out of the cocoon and live in the tube for a short time with the female.

Massachusetts, Connecticut, and in N. Pike's Long Island collection.

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## EXPLANATION OF PLATES.

### Plate III.

Fig. 1. *Micaria longipes*, 1*a*, maxillæ and mandibles, from below; 1*b*, palpus of male; 1*c*, scale from front of abdomen; 1*d*, 1*e*, white scales from spots on abdomen; 1*f*, scale from hinder half of abdomen; 1*h*, epigynum.

Fig. 2. *Micaria montana;* 2*a*, epigynum.

Fig. 3. *Geotrecha bivittata*, ventral view; 3*a*, dorsal view; 3*b*, 3*c*, palpi of male; 3*d*, epigynum; 3*e*, hair of abdomen.

Fig. 4. *Geotrecha pinnata;* 4*a*, epigynum.

Fig. 5. *Geotrecha crocata*, dorsal view; 5*b*, 5*c*. palpi of male; 5*d*, epigynum.

Fig. 6. *Prosthesima atra;* 6*a*, ventral view; 6*b*, 6*c*, palpi of male; 6*d*, epigynum; 6*e*, foot of first pair; 6*f*, foot of fourth pair; 6*g*, hair of abdomen.

Fig. 7. *Prosthesima ecclesiastica;* 7*a*, ventral view; 7*b*, epigynum: 7*c*, 7*d*, palpus of male.

Fig. 8. *Prosthesima depressa;* 8*a*, epigynum.

### Plate IV.

Fig. 1. *Pœcilochroa variegata*; 1*a*, ventral view; 1*b*, 1*c*, palpus of male; 1*d*, epigynum.

Fig. 2. *Pœcilochroa montana*; 2*a*, epigynum.

Fig. 3. *Pœcilochroa bilineata;* 3*a*, ventral view.

Fig. 4. *Gnaphosa conspersa*; 4*a*, ventral view; 4*b*, end of mandible; 4*c*, outer side of male palpus; 4*d*, palpal organ; 4*e*, epigynum.

Fig. 5. *Gnaphosa brumalis*, outer side of male palpus; 5*a*, palpal organ; 5*b*, epigynum.

Fig. 6. *Pythonissa imbecilla;* 6*a*, ventral view; 6*b*, mandible; 6*c*, palpus of male; 6*d*, epigynum.

Fig. 7. *Drassus saccatus;* 7*a*, ventral view; 7*b*, outer side of male palpus; 7*c*, tibia and tarsus of male palpus, under side; 7d, epigynum.

Fig. 8. *Drassus robustus;* 8*a*, epigynum; 8*b*, *c*, palpus of male.

### Plate V.

Fig. 1. *Clubiona crassipalpis*, male palpus showing palpal organ; 1*a*, tibia of male palpus; 1*b*, outer side of male palpus.

Fig. 2. *Clubiona mixta*, male palpus, upper side showing tibia; 2*a*, under side, showing palpal organ; 2*b*, outer side of patella and tibia.

Fig. 3. *Clubiona tibialis*, male palpus, upper side; 3*a*, outer side; 3*b*, epigynum.

Fig. 4. *Clubiona canadensis*, male palpus, upper side of tibia and tarsus; 4*a*, outer side of male palpus; 4*b*, under side of tibia and tarsus, showing palpal organ; 4*c*, epigynum.

Fig. 5. *Clubiona pusilla*, under side of male palpus, showing palpal organ; 5*a*, upper side, showing form of tibia; 5*b*, inner side.

Fig. 6. *Clubiona rubra*, male palpus of spider from Saugus, Mass.; 6*a*, outer side; 6*b*, palpal organ; 6*c*, epigynum.

Fig. 7. *Clubiona rubra*, palpus of large male from Lynn, Mass.; 7*a*, outer side; 7*b*, palpal organ; 7*c*, epigynum.

Fig. 8. *Clubiona rubra*, palpus of small male from New Haven, Conn.; 8*a*, outer side; 8*b*, palpal organ.

Fig. 9. *Clubiona ornata;* 9*a*, epigynum.

Fig. 10. *Clubiona excepta;* 10*a*, dorsal view; 10*b*, 10*c*, palpus of male; 10*d*, epigynum.

Fig. 11. *Clubiona minuta*, male palpus, upper side; 11*a*, outer side; 11*b*, under side.

Fig. 12. *Chiracanthium viride;* 12*a*, dorsal view; 12*b*, male palpus, outer side; 12*c*, upper side; 12*d*, epigynum.

Fig. 13. *Trachelas ruber;* 13*a*, sternum and mouth part; 13*b*, epigynum; 13*c*, 13*d*, male palpus.

## Plate VI.

Fig. 1. *Anyphæna rubra;* 1*a*, ventral view of abdomen, showing at *x* the tracheal opening; 1*b*, epigynum.

Fig. 2. *Anyphæna incerta*; 2*a*, 2*b*, 2*c*, palpus of male; 2*d*, epigynum.

Fig. 3. *Anyphæna calcarata*, under side of coxæ of male, showing spurs on 3d and 4th pairs; 3*a*, 3*b*, 3*c*, male palpi; 3*d*, epigynum.

Fig. 4. *Anyphæna saltabunda*, male palpus, under side; 4*a*, 4*b*, 4*c*, upper and side views; 4*d*, epigynum.

Fig. 5. *Phrurolithus alarius*, in natural position at rest; 5*a*, light-colored variety; 5*b*, first leg; 5*c*, maxillæ; 5*d*, irridescent scales of abdomen; 5*e*, 5*f*, 5*g*, male palpi; 5*h*, epigynum.

Fig. 6. *Phrurolithus pugnatus;* 6*a*, upper side of male palpus; 6*b*, outer side; 6*c*, epigynum, showing part of palpal organ in one side.

Fig. 7. *Agræca pratensis;* 7*a*, sternum and mouth part; 7*b*, 7*c*, 7*d*, palpus of male; 7*e*, epigynum.

## Plate VII.

Fig. 1. *Cœlotes medicinalis*, enlarged 4 times; 1*a*, epigynum; 1*b*, palpus of male, under side; 1*c*, upper side.

Fig. 2. *Cœlotes longitarsus*, palpus of male, upper side; 2*a*, epigynum.

Fig. 3. *Cœlotes montanus*, epigynum; 3*a*, palpus of male; 3*b*, patella and tibia of male from New Haven, Conn.

Fig. 4. *Cœlotes hybridus*, male palpus, upper side of patella and tibia; 4*a*, under side.

Fig. 5. *Tegenaria brevis;* 5*a*, epigynum; 5*b*, head of female; 5*c*, head of male.

Fig. 6. *Tegenaria derhamii*, enlarged 4 times; 6*a*, palpus of male; 6*b*, tarsus and palpal organ; 6c, epigynum.

Figs. 7, 7*a*, 7*b*. *Cicurina complicata*, male palpi; 7, under side; 7*a*, outer side with large process of the tibia separated from the tarsus; 7*b*, upper side, showing the narrow tarsus and short curved tibia.

Fig. 8. *Hahnia bimaculata*, under side of abdomen, showing trachæl opening and spinnerets; 8*a*, male palpus, under side of tibia and tarsus; 8*b*, outer side of tibia and tarsus; 8*c*, 8*d*, 8*e*, 8*f*, various forms of epigynum.

Fig. 9. *Hahnia cinerea;* 9*a*, male palpus, under side; 9*b*, outer side.

Fig. 10. *Hahnia radula*, maxilla of male; 10*a*, hair of first leg of male.

PLATE VIII.

Fig. 1. *Agalena nævia*, male; 1*a*, common form of male palpus from a specimen from Peabody, Mass.; 1*b*, long tubed variety of palpal organ from Woodbridge, Conn.; 1*c*, outer side of tibia of same palpus; 1*d*, short tubed form of palpal organ of a specimen from New Bedford, Mass.; 1*e*, palpal organ with unusually large tube of a specimen from Providence, R. I.; 1*f*, small palpal organ from Jaffrey, N. H.; 1*g*, outer side of small male palpus from Salem, Mass.; 1*h*, common form of epigynum from female in copulation with the male from which Fig. 1 was drawn; 1*i* and 1*j*, epigynum from Providence, R. I.; 1*k*, epigynum with small process on front edge from Salem, Mass.; 1*l*, 1*m*, 1*n*, epigynum from several large spiders from Massachusetts.

Fig. 2. *Dysdera interrita*; 2*a*, sternum, coxæ, and mouth parts; 2*b*, foot; 2*c*, side of male palpus; 2*d*, palpal organ from front.

Fig. 3. *Ariadne bicolor*, female; 3*a*, male, head, palpi, and front leg; 3*b*, sternum and mouth parts of female; 3*c*, male palpus; 3*d*, front leg of female.

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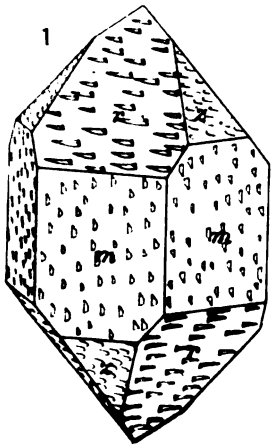

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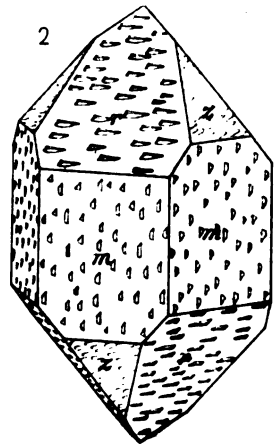

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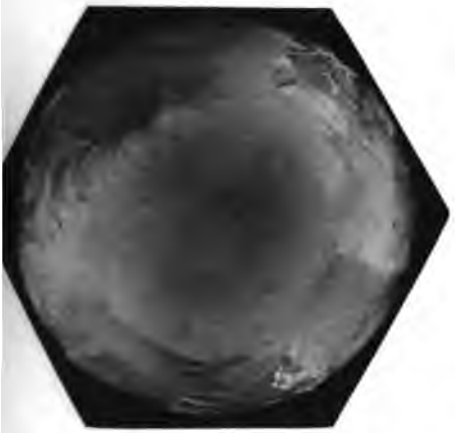

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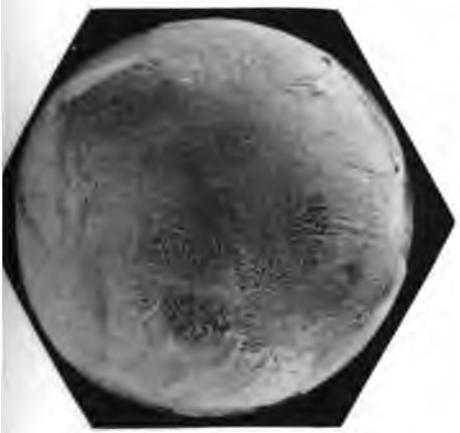

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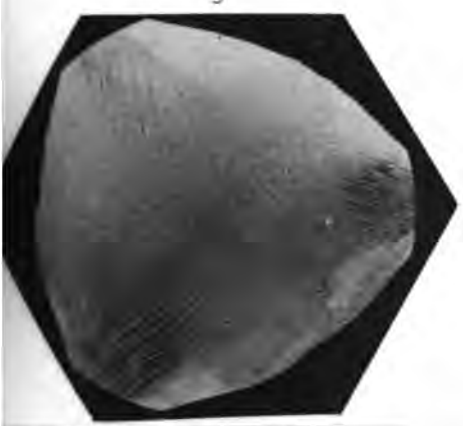

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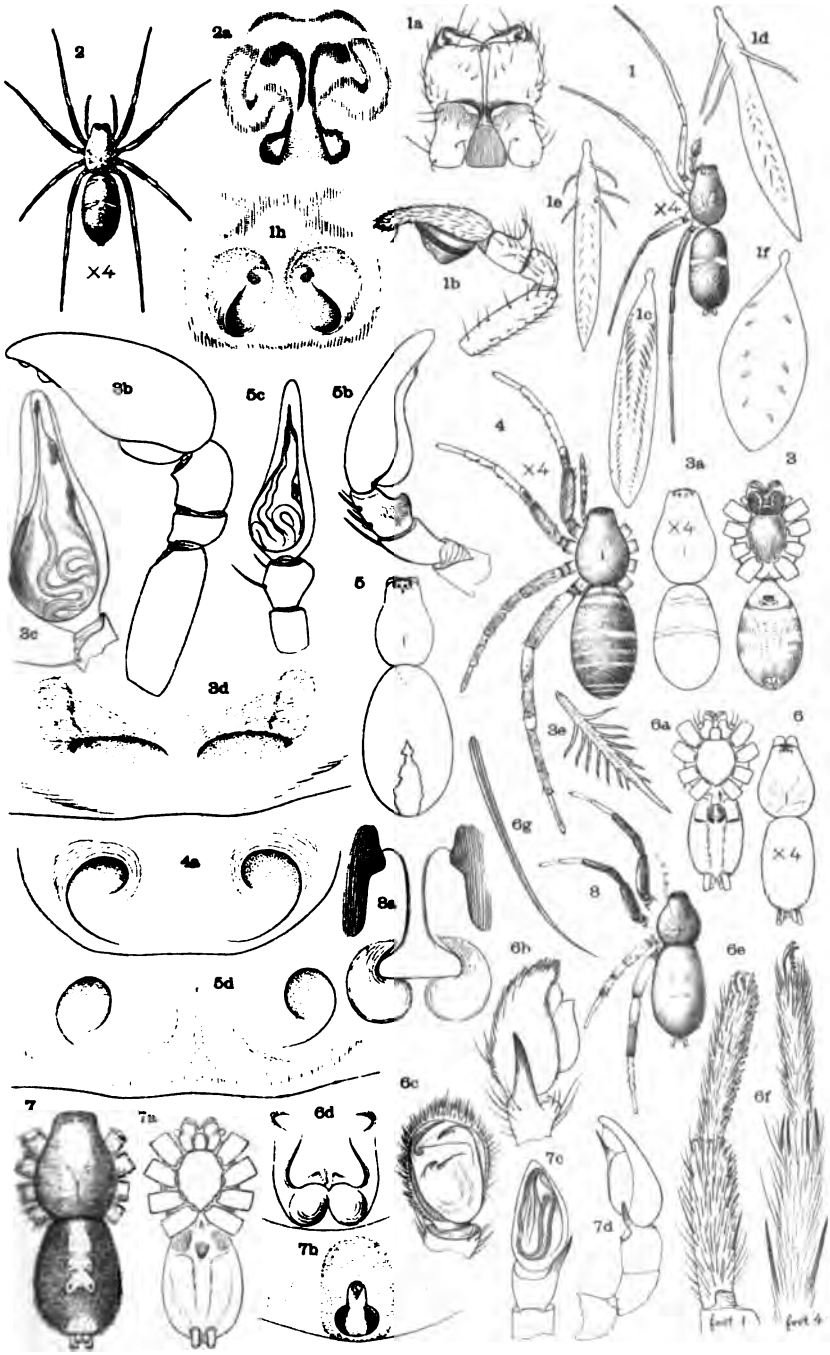


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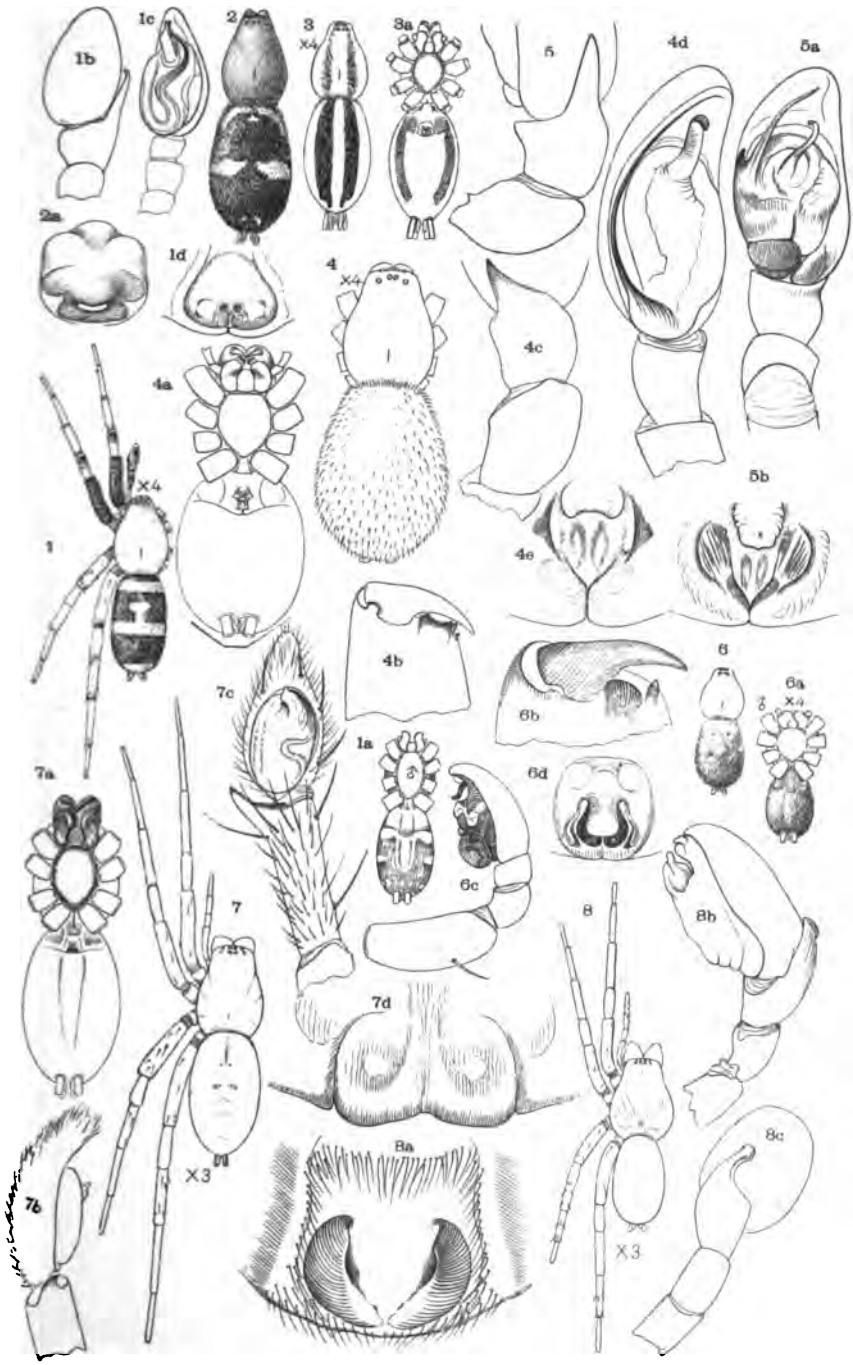


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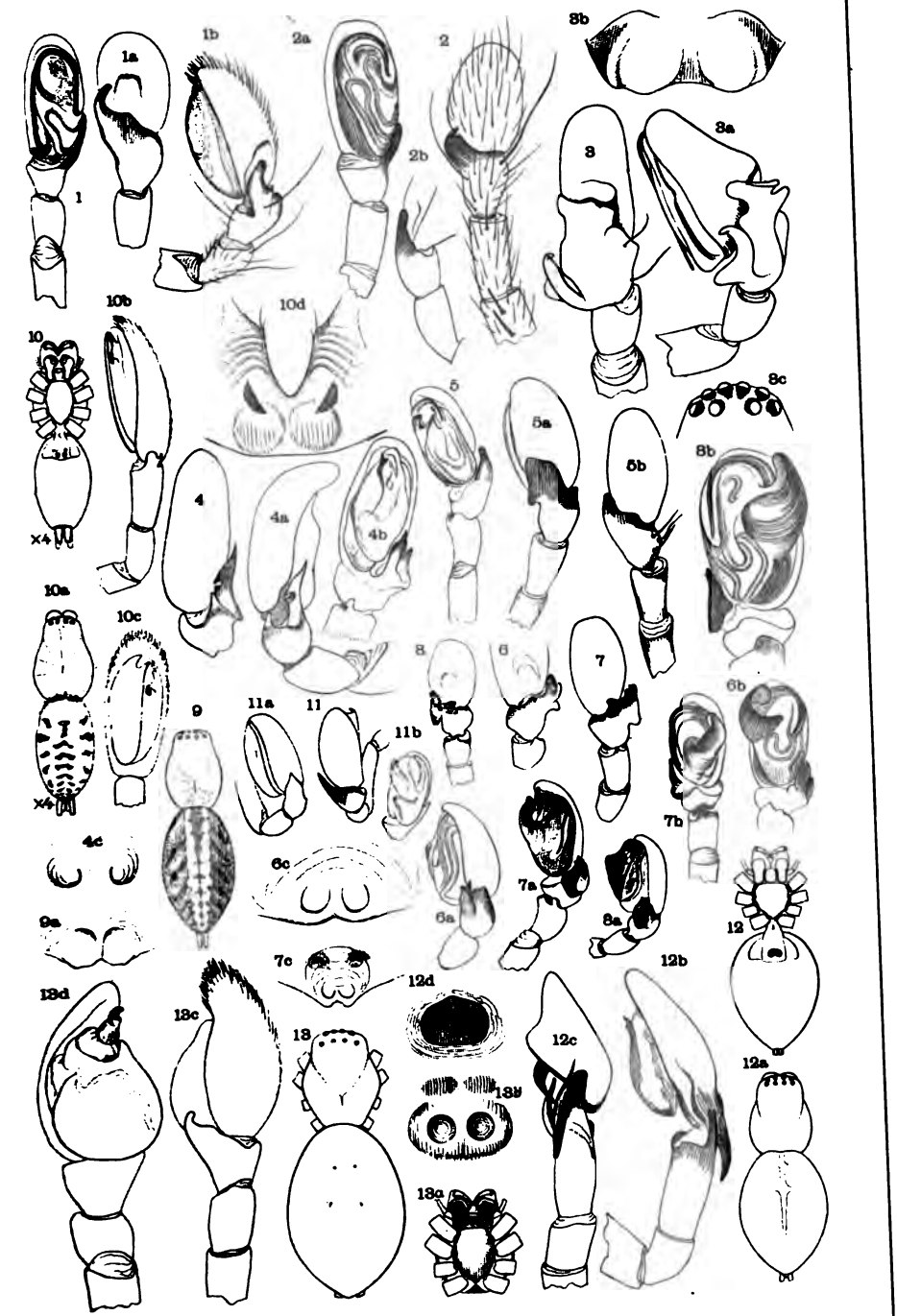


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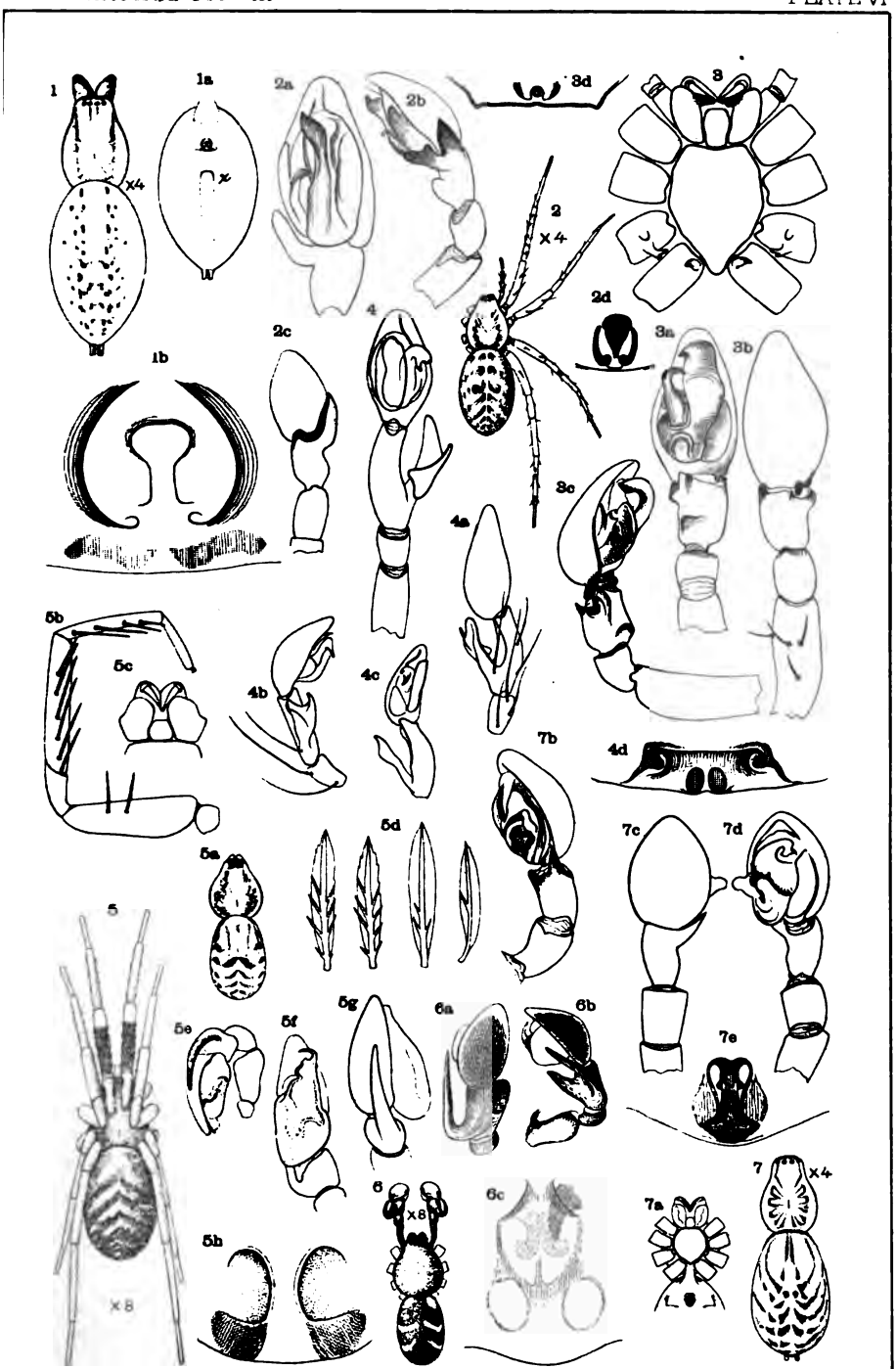


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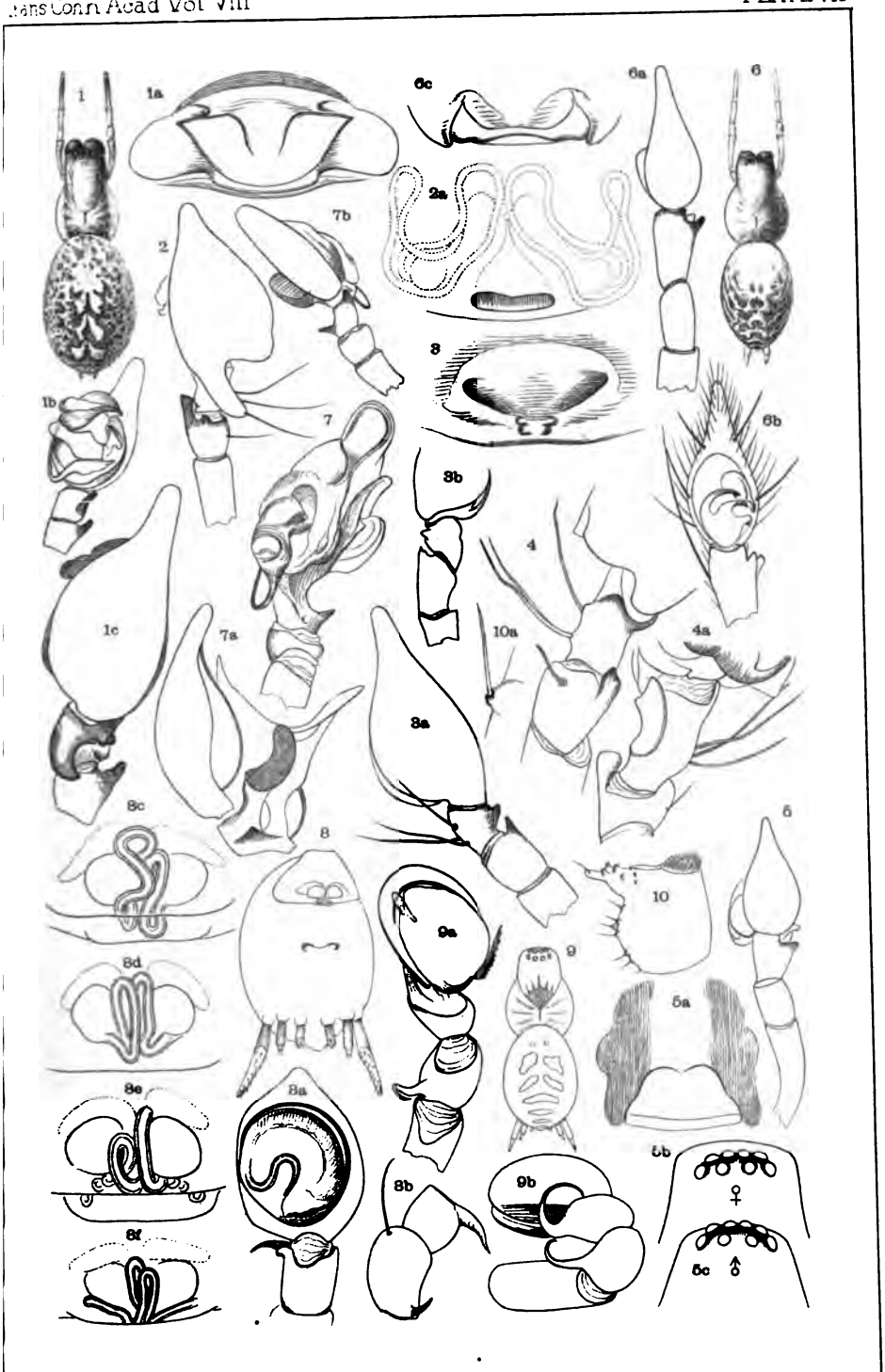


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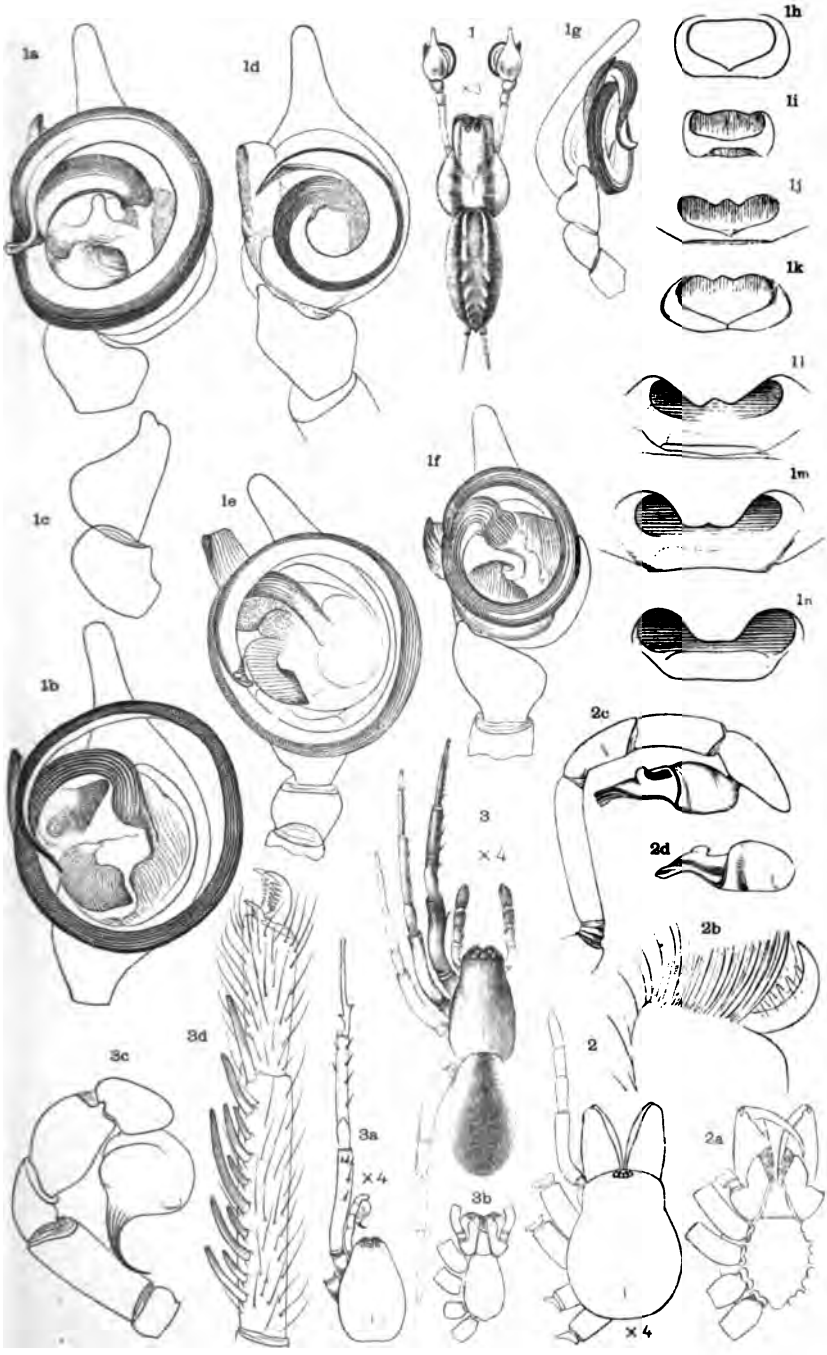


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# TRANSACTIONS

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## VOLUME VIII, PART 2.

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# XII.—The Development of a Paleozoic Poriferous Coral. By Charles E. Beecher. (With Plates IX–XIII.)

The origin and affinities of many groups of paleozoic corals are still obscure. The main elements of the recognized system of classification seem to be stable, yet so little is known of the growth and structure of a number of important groups, that they occupy a different place in almost every arrangement of the genera. Each fact of development affords data which eliminate, to a degree, the want of knowledge concerning their origin and relations. Unless the growth of the organism is obscured by pronounced accelerated or degradational features, its interpretation is simple, and throws much light on its ancestral history. Paleozoic types in general are least modified in their development by acceleration. They usually show some marked expression of their prototype, and also the succession of changes through which they have passed during their evolution.

The species here discussed was originally described as *Michelinia lenticularis*, Hall,* from the Lower Helderberg group of New York. If *Michelinia* is entitled to recognition, it will exclude this form, as it is without tabulæ. *Pleurodictyum*, as now defined, is more in harmony with these features, and, therefore, the species *M. lenticularis* is here referred to this genus. The large calices and their constant origin at the basal epitheca are not, however, essential characters of *Pleurodictyum*. The structure and growth of this species indicate that it represents one of the simpler types of poriferous corals. For this reason, its development is without the numerous modifications necessary in more complex forms, and its laws of growth are not complicated.

*Development of Pleurodictyum lenticulare.*—The nepionic stage is well marked. It comprises the growth of the corallum to the completion of a simple initial cell. This primitive cell or nepionic stage (Plate IX, figure 1, 7, 8) has the form of an oblique inverted cone flattened on one side. The flattened area represents the lower or attached side, and the oblique base of the cone is occupied by the

* Twenty-sixth Rept. N. Y. State Museum of Nat. Hist., p. 113, 1874.

aperture of the corallite. The apical portion is smooth for about one-fourth the length of the cell. Then the concentric lines of growth become apparent, and over the distal half, radiating ribs are also developed. The interior of the apex is granulose. At about the middle of the cell, the granules are arranged in rows, forming the beginnings of the septal lines.

The simple growth of the initial cell continues until the entire procumbent portion is completed. A thickening of the margin then takes place, and an upward growth of the corallite is initiated. At the commencement of this upward growth, the first bud starts out from the lateral edge of the initial calyx, either to the right or left of the axis. This condition represents the first nealogic stage. The bud resembles the parent cell in all particulars, and reaches considerable size before the second appears, as shown in Plate IX, figures 9, 10. The visceral cavities are confluent, as the initial apex of the bud opens into the calyx of the first cell.

The succeeding nealogic stages, to the completion of the first circle of peripheral calices, have been observed mainly from the epithecas of mature or nearly full grown corallums, represented on Plate X, figures 1, 2. In these examples, the lines of growth are so perfectly shown, that all the stages are distinctly marked, and may be satisfactorily studied.

What is here considered as the second nealogic stage is represented on Plate IX, figure 3, showing the initial corallite, with the first and second buds on opposite sides. This process of alternate gemmation from the parent cell continues until the circlet of calices is completed, as shown in figures 4, 5, and 6. In this species, the normal number of peripheral calices is seven, making eight corallites in the completed nealogic corallum. The last cells to be formed are (1) the sixth and seventh budding from the anterior side of the first calyx, and (2) the eighth or posterior cell. Plate IX, figure 12, represents the completed nealogic corallum, with the initial cell and six well developed peripheral calices. The eighth has just begun to fill up the space between the second and third. It will be noticed that there is a direct correspondence in the size of the calices to their relative age. The first calyx is much the largest. Then, decreasing serially, come the second, third, fourth, fifth, sixth, and seventh, while the eighth is undeveloped. An inspection of the upper surface of a mature corallum will thus usually determine the order of successive calical additions. After the appearance of the posterior, or eighth calyx, the corallum commonly grows to double

the diameter of the completed nealogic stage, resulting in the normal ephebolic or mature condition, as represented on Plate XI, figures 1, 2. Nearly all the full grown specimens found agree in this respect.

A corallum rarely presents any departure from the normal number of calices. Plate XII, figure 1, is an example of a variation in the number of peripheral corallities, for in this specimen, there are eight in the circle, instead of the usual seven. A variation in the opposite direction is shown in another specimen having five well developed corallites about the parent cell. Old age characters are expressed in two ways: First, the cell walls become thickened around the margin of the epitheca without destroying the symmetry of the corallum, as shown in Plate XII, figure 2; Second, by the indefinite and unequal development of the peripheral cells, together with the addition of calices budding from the cells forming the primary circle. One specimen, appearing at first sight as an example of cell division or fission, is shown in Plate XIII, figure 2. It may be explained as resulting from the abnormal growth of the second and adjacent calices, four and eight. This lateral impulse further resulted in sending off the small, peripheral, tertiary corallites numbered in the figures 9, 10, 11, 12, and 13.

It should be understood that this arbitrary expression of normal and abnormal growths applies only to the species *P. lenticulare.* The same numerical arrangement will not hold good for genera like *Favosites*, *Michelinia*, *Striatopora*, etc. Otherwise, it is believed, the general laws of growth here brought out will hold good for these and other related genera.

Some doubt may exist as to the propriety of referring the specimens illustrated on Plate IX, figures 9–11, to *P. lenticulare.* Unfortunately, material of this kind is rare and difficult to obtain. With the exception of the position and direction of the first bud (figure 10), all the characters agree, so far as can be observed, with ordinary specimens of *P. lenticulare.* The second cell of the corallum represented in Plate X, figure 1, curves rapidly backwards, although at first the axis has an anterior direction. Taking this view of the specimen, Plate IX, figure 11, it is not difficult to see how the succeeding enlargement and curvature of the bud could extend backwards, thus properly limiting the size of the eighth or last of the primary circlet of calices.

The method of determining the relative age and succession of the corallites can be seen in Plate X, figures 1, 2, and Plate XI, figure 2.

The initial cell occupies the central position, and forms the boss or apex of the basal epitheca. The first bud is nearly on a plane with the base of the initial cell and is the one nearest the apex. The second and successive buds are respectively more distant, and at a higher level. Specimens having broad surfaces of attachment to foreign objects have these distinctive features of the epitheca obliterated, and the only guide to the order of the corallites then lies in their comparative size and position on the upper surface of the corallum.

*General conclusions.*—The first feature to be noted in the development of a poriferous coral, as here described, is the simple cyathiform character of the initial corallite. This nepionic stage is without mural pores, and has an epitheca over the entire exterior of the cup. The septal lines become developed toward the end of this stage. These features are in harmony with the young of many paleozoic corals, such as *Cladochonus*, *Aulopora*, or *Syringopora*, and clearly indicate a primitive, simple, and imperforate ancestry for the Perforata. A similar origin and development obtains in *Favosites*, as may be seen from the figure of a young colony of *F. Forbesi*, var. *occidentalis*, given by Professor Hall.*

The first nealogic stage, represented by the primitive corallite with one bud, is the first transition towards both a compound and a perforate coral, Plate IX, figure 9. This stage has two calices, making it a compound coral, and has an opening through the cell walls or connecting channel between the corallites, forming the first mural pore. The manner of growth and the structure of the corallum at this stage are suggestive of *Aulopora*, and should be given considerable significance. The visceral cavities in *Aulopora* are confluent, and rudimentary septa or lines of spinules are often present. *Romingeria* has a growth resembling *Aulopora* and *Syringopora*. It is without pores on the portion where the corallities and buds are free, but when these are in juxtaposition at their bases, mural pores are developed. The upward growth of the initial cell of *P. lenticulare* proceeds but a short distance before the circlet of peripheral corallites is completed. Thus at this stage there are at least seven mural pores opening into the primary calyx. If this tendency to the formation of numerous buds persists throughout the upward growth

* Indiana Geol. and Nat. Hist., 11th Rept. of the State Geologist, pl. i, figure 12, 1881.

of the corallites, the non-development of the buds consequent upon the adjacent living corallites would naturally result in the production of mural pores. The basal epitheca limits the fleshy portion of the organisms, and represents an area unfavorable to the acquisition of food or for the natural development of calices. Therefore, it would prevent both the maintenance of mural pores and the growth of basal buds.*

A *Favosites* in which one or more cells became inactive or dead shows in its subsequent growth the closing over of this area by the budding of the surrounding cells. Each cell is connected with the parent by an apical pore, Plate XIII, figures 3, 4. Without this opportunity to bud afforded by the death of one or more corallites, or by their divergence, the adjacent cells would have developed only mural pores. In the figure of *P. problematicum* given on Plate XIII, figure 2, three of the initial pores are indicated by dotted lines from *p*. No distinction can be made between these and the ordinary pores, except that the latter are usually not as large. This difference in size would be expected, as the primary pore represents the bud which succeeded in producing a corallite ; whereas the other attempts at budding resulted no further than the production of mural pores. The conclusion to be drawn is, that the mural pores in such genera as *Favosites*, *Striatopora*, *Pleurodictyum*, *Michelinia*, etc., are ineffectual attempts at budding, resulting only in the perforation of the cell walls. This explanation agrees with the pronounced and persistent tendency to gemmation characteristic of the genera mentioned. They also represent compound forms having individualized epithecas, and this feature naturally arises from the same system of budding obtaining in the simple corals.

Professor Verrill has shown that the presence or absence of tabulæ is of little or no importance in a natural classification.† Therefore, the non-tabulate feature of *P. lenticulare* is without special consequence in a discussion of the relations of this species with *Favosites*, or other tabulate poriferous genera.

* The presence of basal mural pores or openings through the epitheca has been asserted by Meek and Worthen (Pal. Illinois, vol. iii, p. 409, 1868). The specimens from which this observation was made, are from a friable sandstone, which does not usually preserve minute details with much distinctness. The depressions between the spinules on the septal lines could easily be mistaken in a cast for the fillings of mural pores, and it is believed by the writer, that this interpretation should be given. *P. lenticulare* occurs as calcareous or silicified, and in the condition of casts. No basal mural pores are present. Also, none can be observed in the casts of *P. problematicum*, from Pelm, Germany.

† Am. Jour. Sci., vol. iii, p. 187, March, 1872.

If the preceding interpretations of structure and affinites are correct, a simple, conical imperforate, non-tabulate prototype, or protocorallum, may be assumed for the Madreporaria Perforata. The next derived form, represented by the early nealogic stages of *P. lenticulare*, has the structure and growth of *Aulopora*, and consists of the parent cell with one or more buds. At this stage, which may be called the *Aulopora-stage*, the initial corallite has the same number of mural pores as developed buds, for each bud leads into the parent cell by a basal opening or pore. *Aulopora* may thus be considered as representing a primitive type of a poriferous coral, in which the number of pores in each corallite corresponds to the number of buds given off plus one connecting it with the parent cell. Some species of this genus are free throughout most of their growth (*A. subtennis*, Hall), agreeing closely with the erect growth of *Romingeria* and *Syringopora*. This fact removes one of the important arguments against the relations of *Aulopora* with these genera. The corallites of *Aulopora* usually send off buds before turning out of the common axis of the branch or colony, after which no gemmation commonly takes places. By the explanation here advanced, this lack of a tendency to gemmation in the distal portions of the corallites in this genus accounts for the absence of mural pores when such portions are in contiguity. The periods of gemmation in *Romingeria* are periodic. Several buds, often forming a verticil are given off from the parent corallite. Considerable elongation of the tubes takes place before other series of buds are produced. The budding is prolific at these points, and here also occur the mural pores. The latter are therefore developed when the period of gemmation is in force. If pores are formed elsewhere when the corallites happen to come into juxtaposition, it may possibly be explained as the result of a stimulus produced by the contiguity of the animals. Further observations are necessary to show that pores exist at other places than the bases of the verticils or points where numerous buds are given off and where from crowding the corallites are in juxtaposition.

It therefore seems, that, primarily, the development of mural pores is identical or homologous with the process of gemmation. Whether this cause is operative in such forms as *Columnopora* or *Alevopora* yet remains for investigation. The porous condition of the walls in these genera may be an inherited character without an active exciting cause, or it may be teleologically different.

Yale Museum, New Haven, Conn., May 20th, 1891.

## EXPLANATION OF PLATES.

### PLATE IX.

*Pleurodictyum lenticulare.*

Fig. 1. Lower side of initial cell, or nepionic stage. × 3½.

Fig. 2. Lower side of initial cell with one bud; first nealogic stage. × 3½.

Fig. 3. Initial cell with two buds; second nealogic stage. × 3½.

Fig. 4. Initial cell with three buds; third nealogic state. × 3½.

Fig. 5. Initial cell with four buds; fourth nealogic stage. × 3½.

Fig. 6. Completed nealogic stage; showing initial corallite (1) and seven peripheral corallites (2-8). × 3½.

Fig. 7. Interior of nepionic corallite. × 3½.

Fig. 8. Exterior of same specimen × 3½.

Fig. 9. Upper side of specimen representing first nealogic or *Aulopora-stage*, consisting of nepionic cell and one bud Apex of bud opens into visceral cavity of parent cell. × 3½.

Fig. 10. Profile of same; showing oblique apertures of corallites, and thickened margin of parent cell. × 3½.

Fig. 11. Lower side of preceding. × 3½.

Fig. 12. Upper side of completed nealogic stage; showing inception of eighth cell. × 3½.

Figs. 1–6 are taken from epithecal lines of growth shown in Plate X, figure 2. Remaining figures are from actual specimens. Numbers refer to order of calical succession.

Lower Helderberg Group. *Albany County, New York.*

### PLATE X.

*Pleurodictyum lenticulare.*

Fig. 1. Lower or epithecal side of specimen; showing successive alternate gemmation from parent corallite. × 3½.

Fig. 2. Similar specimen with lines of growth more strongly marked. The order of budding is opposite to that of preceding specimen. × 3½.

Lower Helderberg Group. *Albany County, New York.*

### PLATE XI.

*Pleurodictyum lenticulare.*

Fig. 1. Outline of calices of specimen Plate X, figure 2; showing central primary cell and seven peripheral calices numbered in the order of their development. × 3½.

Fig. 2. The same; side view.

Lower Helderberg Group. *Albany County, New York.*

## Plate XII.

*Pleurodictyum lenticulare.*

Fig. 1. Outline of upper side of specimen with eight peripheral calices. × 3½.

Fig. 2. Upper side of symmetrical specimen; showing general features of calices, mural pores in central corallite, and thickened epithecal border. × 3½.

Lower Helderberg Group. *Albany County, New York.*

## Plate XIII.

*Pleurodictyum lenticulare.*

Fig. 1. Calical diagram of geratologic specimen; showing enlargement of second, fourth, and eighth corallites, and the addition of tertiary cells, forming a second series of peripheral calices. × 3½.

Lower Helderberg Group. *Albany County, New York.*

*Pleurodictyum problematicum.*

Fig. 2. Lower side of cast of corallum with epitheca removed; showing proximal extremities of several corallites. Upper edge of figure represents portion of periphery of corallum. Thus, lower angle of each corallite represents the point of budding from parent cell, and is connected with it by a pore, shown for three of the corallites by dotted lines from *p*. It will be noticed that all the pores in the angles are larger than the others. Otherwise, these and the initial pores cannot be distinguished from the ordinary mural pores between the flat sides of the corallites. × 7.

Devonian. *Pelm, Germany.*

*Favosites epidermatus?*

Fig. 3. Side view of mature corallite with attached intermural bud. Specimen broken from interior of a large colony. × 3½.

Fig. 4. The same front view, with bud removed; showing pore or mural opening (*p*) at lower point of attachment of bud, corresponding to those indicated in figure 2. × 3½.

Corniferous limestone. *Cherry Valley, New York.*

## XIII.—Symmetrical Cell Development in the Favositidæ. By Charles E. Beecher. (With Plates XIV, XV.)

The majority of compound corals included in the *Favositidæ* are composed of polygonal, prismatic cells or corallites in juxtaposition. When, however, these cells become free, their form is cylindrical. The polygonal form of closely arranged cells is therefore explained as the natural result of crowding.

The species *Pleurodictyum lenticulare*, Hall, sp., is an example of simple cell growth and multiplication. In the development of this species, as shown by the writer in the previous paper, the initial corallite is first conical. The growth of a peripheral series of buds results in changing the sub-circular section of the parent corallite into a polygon. The buds are angular on the sides in juxtaposition to the parent cell and adjacent buds, but on the free portion of their periphery they are cylindrical. The subsequent growth of peripheral buds brings the first series wholly within the corallum, and they are then polygonal in section like the parent corallite.

In compact corals with long cell tubes, as *Michelinia* and *Favosites*, there is a maximum limit to the size of the corallites. Thus, the form of the cells which have reached this limit of diametral extension is that of equal hexagonal prisms. This is of course due to the well known fact of six equal tangent circles about a central circle of the same size. Then from crowding, or from the elimination of the interstitial spaces, they assume a regular hexagonal form. The specimen of *Cleistopora geometrica*, illustrated by Edwards and Haime,* represents the maximum size of the cells and their equal development in this species. Although the tubes are not long, the calices are nearly of the same size, and regularly hexagonal.

After the completion of a circle of calices about the parent cell of the corallum, enlargement takes place, (1) by buds from the periphery, and (2) by intermural gemmation. The first is not attended by any phenomena differing from the production of the primary circlet of calices about the initial cell. The second takes place under other conditions, and is the chief method of increase in the growth of large corallums having numerous corallites.

* Monographie des Polypiers Fossiles des Terraines *Palæozoïques*, p. 252, pl. 17, fig. 3. 1851.

The radial arrangement of the tubes in a large hemispherical or cylindrical mass tends to make the axes of the corallites diverge. This divergence can be taken up only by an increase in the diameters of the tubes, or by the addition of new calices between the others. The latter mode is called intermural gemmation. In *Favosites* and allied genera, the maximum size of the corallites is soon reached, and the expansion of the coral is mainly derived from intermural growth. The study of this method of increase, properly begins after one or more rows of calices have been developed about the parent cell, and the calices have reached their full dimensions.

The following description of a symmetrical system of intermural cell multiplication was observed in a hemispherical specimen of *Michelinia convexa*, D'Orbigny, from the Corniferous limestone of the Falls of the Ohio. It shows very clearly the stages of development of the interstitial buds, and their modifications. Other corals were examined to the same end, and were found to agree in all essential particulars, whenever their growth was not irregular from their condition of fixation, or from the excessive development or death of a number of the corallites. An exact number of peripheral buds is not necessary to illustrate the general laws of intermural growth. The buds produced from any given cell cannot always agree with symmetrical method here described, on account of the crowding of similar series from adjacent or neighboring corallites. After eliminating these variations, it was found that the process of intermural gemmation in general is quite uniform, and closely conforms to that in *Michelinia convexa*.

Plate XIV, figure 1, represents diagrammatically the top of a corallum composed of a central parent cell and six equal peripheral buds, making seven nearly equal calices in the corallum. The upward growth of these corallites and the divergence due to the direction of their axes tend to separate them from the parent cell. In consequence of this separation of the corallites, they would naturally assume a cylindrical form, and there would thus appear triangular interspaces between the tangent points of any three adjacent calices. These angles, therefore, afford the only opportunities for the introduction of a set of intermural buds, and their initial triangular form is determined by the conditions of growth. The smallest number of buds which can be symmetrically placed and compensate for the divergence of the corallites is three, one from each alternate angle of the hexagon, Plate XIV, figure 2.

If these interstitial cells were to grow without the introduction of others, until the original peripheral series was completely separated from the parent or central cell, there would result a corallum containing only triangular corallites. There is, however, a manifest tendency of the organism to the production and maintenance of a cylindrical form, or of a prism with nearly equal radial axes, as in a hexagonal or polygonal prism. To accomplish this, and further to take up the divergence of the corallites, three new interstitial buds are introduced at the remaining three unmodified angles, as shown in figure 3. At this stage, there are six symmetrically disposed triangular buds, or intermural cells, about the central corallite, truncating its original angles, and making it a twelve sided prism. This stage is the third toward the formation of a series of mature interstitial calices.

During the third stage, the intermural buds increase in size until they completely surround the parent cell. Then further growth truncates their adjacent angles, thus adding two more sides to each bud, making them pentagonal in section. This marks the fourth stage of intermural growth. At the same time, the central corallite loses six of its sides, and returns to its early hexagonal form. The axes have revolved 30°, and the original sides have now become the angles of the corallite, Plate XIV, figure 4.

At this period of growth, it is necessary to consider a series of buds on the periphery of the corallum, marked 1″, 2″, etc., in Plate XIV, figures 3 and 4. They are first triangular in form like the others, and of two sizes, owing to their different ages. The growth of this series continues until they touch and truncate the angles of the first series (1′, 2′, etc.), producing the fifth condition or stage. The first series of buds has now three hexagonal and three pentagonal corallites, Plate XIV, figure 5.

In the last or sixth stage, figure 6, the further growth of all the intermural cells results in a corallum of nineteen nearly equal hexagonal corallites. The original parent cell (1) is at the center, the first six intermural cells (1′, 2′, etc.) completely surround it, and the six new peripheral corallites (1″, 2″, etc.) are interposed between the members of the original circlet (1, 2, etc.). The effect of this intermural growth, then, is to dissociate all the first series of corallites from the parent cell and from each other.

The changes taking place in the number and form of the cells may be tabulated as follows :

| Stages. | Form of primary cell. | Whole No. of cells. | Number of intermural buds. | Number of sides of buds. |
|---|---|---|---|---|
| Nepionic. | cone. | 1 | 0 | 0 |
| First completed nealogic or first condition requisite to intermural gemmation. | 6 sided prism. | 7 | 0 | 0 |
| 2d stage. | 9 sided prism. | 11 | 3 | 3 |
| 3d stage. | 12 sided prism. | 16 | 9 | 3 |
| 4th stage. | 6 sided prism. | 19 | 12 | 6 -- 3<br>6 -- 5 |
| 5th stage. | 6 sided prism. | 19 | 12 | 3 ---- 3<br>3 ---- 4<br>3 ---- 5<br>3 ---- 6 |
| 6th stage. | 6 sided prism. | 19 | 12 | 6 |

Buds are developed in *Favosites* and *Michelinia* whenever there is a space or opportunity for their growth, unless the corallum is affected by some abnormal condition. If this tendency to form a solid mass of corallites were not so strong, and if the process of budding took place only at comparatively remote intervals, the corallum would have the form of *Romingeria.* It is evident in *Michelinia convexa,* that if the divergence of the corallites was considerable and not wholly filled by intermural growths, there would result a verticil of corallites about the parent cell which would soon become free. The peripheral corallites, also, would become separated. Then after further growth, the parent cell would give off another verticil of buds, the other corallites, likewise, develop similar verticils, and the whole form and mode of growth be like that of *Romingeria.* From this point of view, *Romingeria* may represent an early form of symmetrical cell development in the poriferous corals. The acceleration of the periods of gemmation, and consequent approximation of the corallites carrying their verticils of buds, would produce all the conditions of cell growth and intermural gemmation exhibited by *Favosites* or *Michelinia.*

SUMMARY :—The growth of intermural buds compensates for the natural divergence of the corallites. New cells are introduced whenever the old corallites have reached their maximum size, and when their divergence approaches a separation of the cell tubes.

The form of the buds is first that of a triangular pyramid or prism, and is due to the mechanical conditions of growth. During subse-

quent increase, they touch and truncate each other, changing from triangular to five- and six-sided prisms. Complete symmetrical normal development produces a corallum with equal hexagonal calices. The process of intermural gemmation changes the sides of the parent cells to angles, and the older corallites, originally in juxtaposition, become separated from each other by new series of interstitial calices.

Yale Museum, New Haven, Conn., May 20th, 1891.

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## EXPLANATION OF PLATES.

### Plate XIV.

*Michelinia convexa.*

Fig. 1. Diagrammatic representation of upper surface of corallum; consisting of parent cell, A, and six peripheral corallites, 1, 2, 3, etc.

Fig. 2. The same; showing the introduction of three triangular intermural buds, 1′, 2′, 3′, etc.

Fig. 3. Third condition; with six triangular buds about the parent corallite, and three on the periphery of the corallum.

Fig. 4. Top of corallum; showing further growth of preceding corallites, with the addition of three peripheral calices, 4″, 5″, 6″.

Fig. 5. The same during a succeeding stage; showing increase in size of corallites, and modifications produced.

Fig. 6. Completed growth of first system of intermural gemmation; showing dissociation of original series of corallites (A, 1, 2, 3, etc.), and representing condition preparatory for new series of interstitial corallites.

All figures natural size.

### Plate XV.

*Michelinia convexa.*

Fig. 7. Development of a group of corallites from initial conical cell to corallum with nineteen calices. The figure represents parallel horizontal sections through the corallum; showing the number and form of the calices, their order of development, and the modifications taking place during growth. The parent cell is marked A; first series of calices, 1, 2, 3, etc.; first series of intermural buds, 1′, 2′, 3′, etc.; peripheral series, 1″, 2″, 3″, etc. Notation corresponds with that of preceding plate.

Natural size.

## XIV.—New England Spiders of the family Attidæ. By J. H. Emerton.

The Attidæ are distinguished by a peculiar arrangement of the eyes. The front of the head is wide and square and the front row of eyes directed forward and nearly straight or with the lateral pair a little the highest. The front middle pair are larger than the others and often much larger, so that at first sight the spider appears to have but two eyes. Behind the lateral eyes of the front row are two very small eyes and still farther back, often near the middle of the cephalothorax, are two others a little larger, sometimes as large as the front lateral pair. See figures on Plates XVII and XVIII.

The relative length of the legs is very variable. The fourth pair is usually the longest, but often the first, and even in some species the third pair. The legs of the first pair are usually thickened, and often those of the second pair. Most species can jump a considerable distance and this seems to be done from all the legs at once and does not depend on their relative length or size. The feet have two claws, generally long and with many small teeth, and, under the claws, a bunch of long hairs. The colors of the Attidæ are generally bright and of great variety. They are partly caused by colored scales and hairs which in some species cover the whole body and in others are so small that the color of the skin shows between them. The scales are usually long and narrow like flattened or branched hairs, Pl. XVI, figs. 1*j*, 1*k*, others, especially those forming the white spots, are short and flat, fig. 5*d*. Some of the scales of many species have a metallic luster and their color changes with the direction of the light. As spiders become older part of their scales rub off and so change their color. In alcohol great changes in color take place. The wetting of the scales makes them transparent and the colors of the skin show through them. For this reason the front of the head and around the eyes is black or dark colored in most species in alcohol while the same parts in life are covered with white or light colored scales. Pl. XVII, figs. 2, 2*a*. Some species that are yellow or brown when alive turn red in alcohol as *Dendryphantes militaris* and *æstivalis*.

The northern Attidæ, like the Drassidæ, are generally of middle size, none of them being as large as the larger Lycosidæ and Epeiridæ,

or as small as a large part of the Theridiдæ. Some species live on the ground and under stones and leaves but most of them on plants and in open places. They make no webs except nests in which they hide in winter, or when moulting or laying eggs. The pairing of some species takes place in the nest, and the males of several of them enter the nest of a young female and wait for her to mature. The habits of the Attidæ, especially the fighting and mating habits of many species, have been described by G. W. and E. G. Peckham, in the papers of the Wisconsin Nat. Hist. Society of 1889 and 1890.

The palpi of the males are less variable than in most families. They are usually stout and short with a short tibial hook and short tube to the palpal organ, the end of the tube resting in a groove in the end of the tarsus. The end of the tarsus is obliquely flattened and covered closely with short fine hairs.

A large number of the American Attidæ have been described. Hentz described some forty species under fifty-one names. Many of these are comparatively easy to identify but as with other families probably a quarter of them will always remain uncertain.

The species described by Walckenaer and Koch are even more uncertain than those of Hentz. A considerable number of them are very probably the same as species described here and in the papers of Mr. Peckham, but the descriptions and figures are in most cases too indefinite to be identified.

The spiders described by Keyserling, in 1885, are, most of them, in the Museum of Comparative Zoölogy, at Cambridge, Mass., and I have examined and identified most of them. More species have lately been described by G. W. Peckham and Mrs. Peckham, who have made a specialty of this family, in the Transactions of the Wisconsin Academy of Sciences for 1888. I have compared specimens of these species with my own. I have also had for comparison a number of European Attidæ named for me by Mr. Simon. The classification of the American *Attidæ* needs a more thorough revision than can be undertaken in a paper dealing with so small a number of species as the present and it will no doubt soon be done by Mr. Peckham. The classification of Simon as modified by Peckham has been followed as far as possible.

The species are included in the following genera:

*Phidippus* Koch. Large hairy spiders. Mandibles large and strong and longer than the front of the head. Cephalothorax widened across the middle. Anterior row of eyes comparatively small and a little separated from one another, the lateral higher than

the front pair. Middle eyes nearer the lateral than the dorsal. Legs short and stout 4, 1, 2, 3 in females and 1, 4, 2, 3 in males. Males with longer legs and deeper colors than females. Male palpi with a short tibial hook and short tube of the palpal organ. · *P. rufus, ruber, brunnens, mystaceus, tripunctatus*, page 224 to 228.

*Dendryphantes* Koch. Hairy spiders of moderate size. Front row of eyes large and short distances apart, the lateral only a little higher than the front pair. Middle eyes half way between the lateral and dorsal or a little nearer the lateral. Mandibles large and strong, those of the males spreading a little apart and having a sharp edge on the outer side. Legs 4, 1, 2, 3 in females and 1, 4, 2, 3 in males. Colors of the sexes very different. Females brown and gray, indistinctly marked. Males darker brown with bright white markings, color of skin changing to red in alcohol. *D. militaris, montanus, æstivalis*, page 228 to 230.

*Icius* Simon. I use this genus as it has been used by Keyserling and Peckham to include several species that must soon be separated into two or three genera. *I. mitratus* and *palmarun* resemble *Dendryphantes*, but have the cephalothorax more flattened, the abdomen narrower and longer, and the front legs of the males more elongated. The other species approach *Epiblemum* and *Menemerus*. They have the cephalothorax elongated, the colors dark and the hairs and scales very small and partly iridescent. The legs are marked with longitudinal lines and the front pair much elongated. *I. palmarum, mitratus, elegans, hartii, formicarius*, page 232 to 236.

*Marptusa* Thorell = *Marpissa* Simon. Cephalothorax and abdomen much flattened and both widest across the middle. Cephalothorax very low in front, the front eyes touching its lower edge. Lateral eyes half their diameter from the front pair. Mandibles small. Legs 4, 1, 2, 3, in females and 1, 4, 2, 3, in males. *M. familiaris*, p. 237.

*Epiblemum* Hentz = *Calliethera* Koch and Simon. Cephalothorax more than half longer than wide, a little widened in the middle and depressed in the middle between the cephalic and thoracic parts. Abdomen oval, not much longer than the cephalothorax. Legs 4, 1, 2, 3, in females and 1, 4, 2, 3, in males. Mandibles of the males elongated and turned forward. Distinct white markings on cephalothorax and abdomen. *E. scenicum*, page 238.

*Menemerus* Simon. Cephalothorax half longer than wide, widest a little behind the middle, flattened above, and with a deep groove across the middle. Legs 4, 1, 2, 3, or 1, 4, 2, 3, front legs thickened. Colors in longitudinal stripes. *M. binus, lineatus*, page 239.

*Mævia* Koch. Cephalothorax high and wide, but little widened in the middle. Mandibles small. Legs long, slender and tapering, differing little in length, 4, 1, 2, 3. Hairs and scales short and colors bright. *M. vittata*, page 236.

*Zygoballus* Peckham. Cephalothorax high. Eye space nearly as long as wide and widest behind. Dorsal eyes very high and wide apart. Mandibles large in both sexes, those of males with spines on the inner and under sides. Legs 4, 1, 2, 3 and 1, 4, 2, 3. *Z. bettini*, *terrestris*, page 230, 231.

*Phlegra* Simon. Cephalothorax long with the cephalic part short, about half as long as the thoracic. Lateral eyes higher than the front pair. First and second legs short and thickened. Legs 4, 3, 1, 2 or 4, 1, 3, 2. Markings in longitudinal lines. *P. leopardus*, page 242.

*Hasarius* Simon. Cephalothorax a third longer than wide, widest in the middle and flat above. Abdomen larger than cephalothorax, widest across the middle, and pointed behind. Dorsal eyes as far apart as lateral. Legs of third and fourth pairs of equal length. First or fourth pair longest. The cephalothorax has usually a band of light color across the middle behind the eyes. *H. hoyi*, page 243.

*Habrocestum* Simon. Cephalothorax high, longer than in Attus and the dorsal eyes farther back. Colors black and white in females and bright and iridescent in males. Third legs longest. *H. splendens*, *peregrinum*, page 244, 245.

*Attus* Walck., Simon. Cephalothorax nearly as wide as long, widest in the middle. Eye space two-thirds as long as wide. Dorsal eyes as far apart as the lateral. Third legs shortest. Fourth legs longest in females and usually in males. *A. palustris*, *sylvestris*, page 247.

*Saitis* Simon. Small spiders with the cephalothorax long and widest behind. Dorsal eyes nearer together than the front lateral. Abdomen short and wider than the cephalothorax. Third legs as long as fourth and longer than first. *S. pulex*, page 246.

*Euophrys* Koch, Simon. Cephalothorax low and wide in front, long and with the sides almost parallel. Legs 4, 1, 2, 3, first and second pairs thickened. Hairs short and skin in the females marked with gray and brown spots like Tegenaria. Males darker colored. Some species with long hairs and scales and markings like *Attus*. *E. monadnock*, page 241.

*Neon* Simon. Small spiders with large eyes; the dorsal pair as large as the lateral and nearer the back than the front of the cepha-

lothorax. Hairs short and skin marked with gray spots. *N. nellii.*, page 240.

The three following genera are ant-like in general appearance. They are long and narrow. The pedicel of the abdomen is long and visible from above. Abdomen and cephalothorax each with a depression near the middle; legs slender.

*Synageles* Simon. Dorsal eyes as far back as the middle of the cephalothorax. Transverse depression very slight. Cephalothorax flattened above, the hinder half narrowing slightly backward. *S. picata*, page 250.

*Salticus* Latreille, Simon. Cephalic part of cephalothorax higher than the thoracic. Mandibles large. Palpi of females with tibia and tarsus thickened. *S. ephippiatus*, page 249.

*Synemosyna* Hentz. Very long and narrow. Depressions in cephalothorax and abdomen deep. Cephalothorax and abdomen both narrowed toward the pedicel. Middle eyes very large; the others all small. *S. formica*, page 248.

## Phidippus multiformis, new sp.

*Phidippus rufus* Peckham, but not *Attus rufus* Hentz, nor *A. castaneus* Hentz, nor *Phidippus ruber* (Keyserling) Peckham. Keyserling has named this species *Phidippus auctus* Koch in the Cambridge museum.

The female is 8 to 9$^{mm}$ long, the male 5 to 7$^{mm}$. The general color of the adult female is yellowish brown with black and white markings. Around the front of the abdomen is a white band, and on the back are two indistinct longitudinal black stripes in which are four pairs of white spots. The general brown color is produced by a mixture of scales and hairs of various colors. The females are most brightly colored just before reaching maturity. The general color is then a bright orange, mixed with darker red and brown scales and black hairs. The cephalothorax is covered with yellow scales inclining to red in the middle of the back. The black stripes on the abdomen are more distinct and the white spots larger. Pl. XVI, fig. 1*b*. The legs are pale in the middle of the joints and dark toward the ends and covered with gray and black hairs. The palpi are yellow. The hairs and scales are of various shapes, the most common being that of slightly flattened hairs, fig. 1*j*. These, with black hairs, form the darker colors. The yellow and orange scales are wider and less sharply pointed, fig. 1*k*, and the white spots have short and wide scales. Under the abdomen the color is light gray with two parallel darker stripes. In younger spiders the black

markings of the abdomen are larger and the white markings of the hinder half larger and wider. The yellow parts are paler and the joints of the legs are less darkened toward the ends. In very young, just after leaving the cocoon, fig. 1*d*, the legs are entirely pale, except the claws, which are black; the cephalothorax is black with a few yellow scales. The abdomen, which is very short, is black with a yellow marking along the middle and around the front half. The two hinder pairs of white spots are twice as wide as long, and the other pair are very small.

In alcohol the orange color disappears almost entirely. The palpi remain light yellow and the colors of the legs become lighter and darker shades of yellowish brown. The cephalothorax is dark brown, darker on the head, and the abdomen becomes brown with the black and white markings still distinct but faded.

The colors of the male are entirely different from those of the female, fig. 1*a*. The cephalothorax and legs to the end of the tibia are black. The metatarsus and tarsus of all the legs are reddish brown with black ends. The palpi are black with a stripe of white scales on the upper side of patella, tibia and half of the tarsus. Abdomen black beneath and in the middle above. Around the front end is a white stripe. The sides of the top of the abdomen are bright orange and between the orange and black areas are three pairs of white spots. In the middle of the black area is a lighter stripe of yellowish iridescent scales which, in some lights, can not be easily seen, and may be absent altogether.

The epigynum has a round opening in front with the sides slightly darkened and toothed. The hinder notch is rounded but variable in shape. Pl. I, fig. 1*e*, 1*f*.

The male palpus has the tibial hook, small and sharp. Fig. 1*h*, 1*i*.

This is one of the most common Attidæ on plants throughout the summer. It matures in July and the males and females may be found together in a bag of silk among leaves, and in the same bag the female makes her cocoon of eggs, from which the young come out in August and become half grown before winter.

Mt. Washington and Dublin, N. H., Eastern Massachusetts, New Haven, Conn.

**Phidippus brunneus**, new sp.

This spider closely resembles *P. multiformis*, and I supposed it to be a variety of that species. The size is the same and it has indistinctly the same markings on the abdomen in some individuals. The differ-

ences are in the color and the epigynum. The color in life is reddish brown, covered with gray and black hairs and small gray scales not close enough to cover the skin. The cephalothorax is a little darker brown than the abdomen. The abdominal markings are indistinct in some individuals, and in most entirely absent. The legs are more uniformly colored than in *P. rufus*, the ends of the joints being less distinctly darkened. The epigynum, Pl. I, fig. 2, is more distinct than in *multiformis*, the dark thickened edges of the anterior opening are longer and the opening is divided in front by a thick brown middle line into two.

Salem and Waltham, Mass.

**Phidippus ruber** Keys. not *P. rufus* Peckham.

This species is known to me principally by the males. These are 6 or $7^{mm}$ long. The cephalothorax and abdomen are bright orange above and black beneath. The abdomen is covered with large orange scales lighter colored than those of the cephalothorax and mixed with the scales are long black hairs. On the hinder half of the abdomen are two distinct black longitudinal stripes in which are two pairs of white spots. Pl. XV, fig. 4. These markings vary in size and in one male are almost covered by orange scales. The femora of all the legs and tibia of first pair are black, the other joints orange brown, darker or black toward the tips. The male palpi have the tibial hook flat and round at the end. Fig. 4*b*, 4*c*.

The female which I suppose to belong to this species is $9^{mm}$ long. The abdomen is covered with orange yellow scales and black hairs finer and shorter than in the male. There are no black stripes. Around the front of the abdomen is a white stripe. The cephalothorax is brown covered with orange scales. The legs are orange brown, the front pair darkest and the femora of all the legs darker than the other joints, all of which are slightly darker toward the tip. The mandibles are brown in both sexes and not iridescent. The sternum of the female is dark, almost black, but the abdomen is as light beneath as it is above. The epigynum has two small openings. Fig. 4*a*.

Female from Providence, R. I. Males, Topsfield, Mass., Sherborn, Mass., and House Island near Manchester, Mass.

## Phidippus mystaceus.

*Attus mystaceus* Hentz, ♀ *Phidippus albomaculatus* Keys., ♂ *P. purpuratus* Keys., *P. galathea* Peckham.

The largest of the New England Attidæ, females measuring 12 to 15$^{mm}$ long. The female is black, covered with gray hairs and scales closely enough to give the whole spider a gray color. On the abdomen are two longitudinal black stripes that do not extend quite to the front end and in these stripes are four pairs of bright white spots. The front end of the abdomen is crossed by a white band that extends back obliquely each side and at the sides of the abdomen are several oblique white stripes with black edges. Pl. XVI, fig. 3. The hairs of the palpi and front of the head under the eyes are longer and whiter than on other parts of the body. Under the abdomen is a middle dark line narrowing behind, on each side of which is a light gray border. At the sides of this are two wider dark bands and outside of them lighter gray. Legs gray, not ringed but a little darker toward the ends of the joints.

In alcohol the general color becomes dark brown and later reddish and all the markings are less distinct.

The male resembles the female but is darker colored. The cephalothorax, palpi and legs are black with a few scattered white scales and hairs. Abdomen black beneath. Back of abdomen with a greenish white border and a black area in the middle in which are four pairs of white spots.

The male palpus is large. The tarsal hook is nearly as long as the tarsus and sharp and slightly curved at the tip. Fig. 3*a*, 3*b*.

The epigynum has a hard plate with two small openings in front and a notch of various shapes behind. Fig. 3*c*, 3*d*.

It lives under stones at all seasons. In winter or when moulting or laying eggs it hides in a thick white bag of silk, in which the cocoons are made early in the summer. The young become nearly full grown before winter. Adult males are found from May to July and adult females at all seasons.

Common all over New England. Peckham has not found it in Wisconsin.

## Phidippus tripunctatus.

*Attus audax* Hentz, *Attus tripunctatus* Hentz, *Phidippus morsitans* Peckham.

A large female from Connecticut, measures 10$^{mm}$ long and they are usually 8 to 9$^{mm}$. Peckham says western specimens are larger, often as long as 15$^{mm}$.

The color of both sexes is black, with black mixed with a few white hairs. The legs are gray in the middle of the joints and black toward the tips. The abdomen has a white band around the front and is marked with three large white spots, the middle one corresponding to the second pair in *multiformis* and *mystaceus* and the other two to the third pair. The other spots are generally present in this species but so small as not to be readily noticed. The under side of the body is black with two indistinct light stripes under the abdomen. The mandibles are metallic green and blue.

The males are 6 or $7^{mm}$ long and colored like the female. The mandibles have a short blunt tooth in front over the claw. Fig. 5*a*.

The male palpi have the tarsal hook pointed and turned downward. Fig. 5*c*. The palpal organ is wider at the base than in most species. Fig. 5*b*.

It lives under bark and stones, hibernating half grown in thick silk nests and concealing its egg cocoons in the same places. It is common all over the United States.

### Dendryphantes æstivalis Peckham, 1883.

*D. capitatus* Peckham, 1885. Resembles *Attus capitatus* and *parvus* Hentz.

Females 5 or $6^{mm}$ long and males smaller. There are two varieties in the colors of the females. The light variety, Pl. XVII, fig. 2, has the light parts white or light yellow and the dark parts dark brown covered with white hairs and scales. The cephalothorax is dark brown thinly covered with scales so that the dark color shows between them in spots. The legs are light yellow and translucent, indistinctly ringed with brown at the base, and near the tip of each joint all covered with greenish white hairs. The palpi are light and without rings except on the femur and patella. Abdomen brighter yellow than the thorax with four pairs of purplish brown spots, the second pair largest, connected with a paler brown middle marking. Abdomen beneath with a purple brown stripe in the middle and oblique brown stripes at the sides. Sternum, maxillæ and mandibles light brown. Scales smaller than those of the dark variety.

The dark variety, fig. 2*b*, is generally smaller and covered with longer hairs and scales. The legs and palpi are more distinctly ringed with dark brown. The dark spots on the abdomen are smaller and more connected by dark lines than in the other variety. The under side is dark brown.

In alcohol they become in a few days bright red in the darker parts which afterward fade and remain dull red for a long time. Both varieties in alcohol look much alike.

One of the most common Attidæ, living on plants, especially evergreen trees, all over New England.

The colors of the male, Fig. 2*c*, differ extremely from those of the female. The legs are ringed as in the female, but the brown parts are wider and not obscured by white hairs, while the white parts are whiter. The cephalothorax is dark brown with a white stripe each side under the eyes, bending toward each other behind but not connected. The front of the head is also white and covered with long white hairs. The palpi have the femur dark brown at the base and white at the end. The patella and tibia are brown and the tarsus is brown with white hairs on the upper side. The abdomen is white in front and around the sides. The middle is dark brown with a few yellow and greenish scales in the middle. The brown area is usually notched at the sides in four scallops and sometimes indistinctly divided in four pairs of spots as in the female.

In the female the cepalothorax is a third longer than wide and not much widened across the middle. In the male the cephalothorax is only slightly wider. The mandibles are vertical in both sexes and but little larger in the male than in the female.

The male palpi and the palpal organs are large in proportion to the size of the spider. The palpal organ extends back beyond the tibia. The tibial hook is very small. Fig. 2*e*. The tube of the palpal organ has a stiff point at its side a little longer than the tube itself. Fig. 2*d*.

The epigynum has the front opening small and divided into two directed sidewise. Fig. 2*f*.

**Dendryphantes montanus**, new sp.

Female $7^{mm}$ long, a little smaller than *militaris*, cephalothorax as long as in *militaris* but not as wide. Legs and mouth parts a little more slender than in *militaris*. The markings, as far as can be judged from specimens in alcohol, are like those of *militaris*.

The male has the cephalothorax nearly as wide as long. Pl. XVII, fig. 3*a*. The mandibles are as long as in *militaris* but not as stout, fig. 3*b*, and the palpi are as long and much stouter. The femora of the palpi are thickened towards the end, as in *æstivalis*, and the tibia and patella are very short. The palpal organ is long, and the tube long and stout. Figs. 3*d*, 3*e*. The epigynum, fig. 3*c*, has a single round opening, and a notch of various shapes, like *æstivalis*.

Mt. Washington, N. H.

## Dendryphantes militaris.

*Attus militaris* Hentz. *Philœus militaris* Peckham.

Female 7 or 8$^{mm}$ long. The cephalothorax is wide across the middle in both sexes, nearly as wide as long. The middle eyes are a little nearer the front lateral than the dorsal eyes. The mandibles are a little flattened in front, and in the male, the front outer corner over the claw is sharp. Pl. XVII, fig. 1*a*.

The general color is brown, covered with black, and a few gray hairs, darker and browner than *æstivalis*. In alcohol it turns red but not so bright as *æstivalis*. The cephalothorax is brown in the female, and in the male the same color, with a white stripe each side under the eyes. Fig. 1*a*. The abdomen of the female is brown, with four pairs of white, oblique marks in the middle, and four at the sides. Fig. 1. The front of the abdomen is white. In the male the middle white spots are absent, and the lateral and front white marks are united into a band that extends nearly around the abdomen. Fig. 1*a*.

The palpi of both sexes are very slender, and the tarsi and palpal organs of the male are unusually small for so large a spider. The tube of the palpal organs is longer than in *æstivalis* and more simple. Fig. 1*d*.

The mandibles of the male are widened at the end, and have a large two-pointed tooth near the end of the claw. The claw has a short, flat tooth on the inner side, near the middle. Fig. 1*b*, 1*c*. The epigynum is like that of *æstivalis*, but the opening is wider.

## Zygoballus bettini Peckham.

A larger and more slender species than *terrestris*. The body is higher and narrower, the legs and palpi are larger, and the complicated mandibles of the male are larger in proportion to their thickness. The cephalothorax is almost as high in the middle as it is wide, and slopes from the hinder eyes steeply backward. Toward the front the slope is less, and the front of the head is half as high as the hind pair of eyes. Pl. XVII, fig. 4. The abdomen is rounded above and widest across the hinder half.

In life, the colors are bronze green and yellow, marked with white. In alcohol the cephalothorax is dark brown with whitish scales. The abdomen is lighter brown, sometimes reddish, especially in specimens not long preserved, with white markings sometimes forming a regular herring-bone figure in the middle, and sometimes broken

into irregular dark and light spots. Around the front is a white band, and there are other slanting white stripes along the sides.

The femur of the first pair of legs is very dark brown, the other joints white. The second and third legs are white, or only slightly darker at the ends of the joints. The fourth legs have the hinder half of the coxæ, and the ends of all the joints brown.

In the male the legs and palpi are brown, without the distinct dark markings of the female. The abdomen is brown with irridescent greenish scales, and a bright, white band around the front, and two white oblique stripes each side.

The male has the front legs much larger than the female. His mandibles are long and stout at the base, and spread apart at the ends. In the middle of the inner side is a large tooth, directed downward, and near its base, two smaller ones, on the under side of the mandible. On the under side, near the outer edge, is a long ridge, ending in a tooth, curved inward. Fig. 4*a*. The male palpi are very slender, and the tarsus and palpal organ long and small. The patella and tibia are each twice as long as thick. The tibial hook is nearly as long as the tibia. Fig. 4*b*.

Common on plants in summer, and occasionally found under stones and bark.

Massachusetts and Connecticut.

**Zygoballus terrestris**, new sp.

Length of female $3^{mm}$. The cephalothorax is two-thirds as wide as long, and half as high as long. The posterior eyes are the full width of the cephalothorax apart, and the thorax slopes backward from them, but not as steeply as in *bettini*, nor is it as much narrowed behind. Pl. XVII, fig. 5.

The color of the cephalothorax in alcohol, is dark brown, covered thinly with small, light colored scales. The first pair of legs are dark brown, the femur darkest, and the other joints a little lighter in the middle. The other legs are lighter with the ends of the joints dark. Fig. 5*c*. The abdomen is lighter than the thorax, and marked with irregular and variable dark spots.

In the male the abdomen is brown in the middle, with a distinct white line around the front and sides. The male palpi are short, the patella and tibia being not much longer than wide. The tibial hook is as long as the tibia itself, and only slightly curved. Fig. 5*d*. The palpal organ is small and covered by the tarsus. The tube is moderately long, and lies in the groove at the end of the tarsus for

its whole length. The mandibles are similar to those of *bettini*, but smaller. The epigynum has the anterior opening nearly square, opening toward the front, and the posterior notch very wide. Fig. 5*b*.

On fences and under leaves in winter.

Boston and Cambridge, Mass., and New Haven, Conn.

### Icius mitratus Peckham.

*Attus mitratus* Hentz.

This species resembles closely *palmarum*, differing mainly in color. The legs are all white in both sexes, and the mandibles of the male are not long and horizontal as in *palmarum*. The male palpi and palpal organs are like those of *palmarum*, with the tibial hook perhaps a little more slender. The female preserved in alcohol is still more like *palmarum*. The epigynum is of the same shape and the markings in four large spots on the abdomen, like those of some females of *palmarum*. The cephalothorax seems to be a little wider, and the colors of the hairs of the whole body whiter than in *palmarum*.

A living male has the legs white, or a little greenish, with long white hairs, those on the front legs longer than the diameter of the leg. On the front of the leg and palpi are long, white hairs. The sides of the cephalothorax and abdomen, and the under side of the abdomen are white. The middle of the cephalothorax and abdomen are light brown, covered with light, yellow hairs, through which three or four dark spots show indistinctly. Pl. XVIII, fig. 2.

Brookline and Malden, Mass., and New Haven, Conn.

### Icius palmarum Peckham.

*Epiblemum palmarum* Hentz.

Female $5^{mm}$ and male $4^{mm}$ long. The living female has the legs and palpi transparent white, and the claws black. The few spines on the legs are black. The whole body is covered with light gray or white scales, mixed with short, fine black hairs. The abdomen has a row of indistinct, darker triangular spots in the middle and oblique rows of small spots at the sides. Pl. XVII, fig. 1. In alcohol the legs become dull yellow, and the rest of the body turns red as in *æstivalis*, afterwards fading to a dirty yellow. The markings of the abdomen become more distinct, and in some individuals form four large, dark brown spots. Fig. 1*f*.

The living males, fig. 1*g*, have the front legs very dark brown, except the tarsi, which are a little lighter. The other legs are transparent white, cephalothorax and abdomen dark and reddish brown, mixed with shining, greenish white scales, and sometimes a little copper red around the eyes. There is a white stripe the whole length of the body, each side, and across the front of the head below the eyes. The maxillæ and mandibles are dark brown, and the palpi are the same color, except the tarsi which are light yellow ; sternum and under side of abdomen dark brown. Some males show indistinctly dorsal markings of the abdomen, like the female.

The female resembles *æstivalis*, but is longer in proportion to its width and has the front legs stouter. The abdomen is usually nearly twice as long as wide. Fig. 1. The cephalothorax is flattened above from the front eyes two-thirds its length backward, in both sexes, fig. 1*a*, while in *æstivalis* it is slightly arched upward.

In the males, the front legs are, as usual, longer and stouter than the others and are made very conspicuous by their dark color. The mandibles of the male are larger than those of the female, and more or less turned forward, according to their length. In some males the mandibles are only a little longer than those of the female, and in these the patella and tibia of the front legs are not much longer than the femur. Others, usually larger spiders, have the mandibles nearly as long as the cephalothorax and extending forward horizontally, the maxillæ are longer, and the first pair of legs have the patella and tibia one and a half times as long as the femur. Fig. 1*c*. Fig. 1*g* shows the mandibles of the common length.

The epigynum has two small anterior openings and a large, wide notch behind, half-way between the anterior openings and the transverse fold.

The male palpus has the tibia as wide as long, and the patella a little longer. The tibial hook is as long at the joint itself, thin and curved inward a little at the end. The tube of the palpal organ is long and curved around the end of the bulb, the point resting in a groove turned obliquely outward on the end of the tarsus. Figs. 1*h*, 1*i*.

On plants in summer. Massachusetts and Connecticut.

**cius elegans,** ♀ *Attus elegans* Hentz. ♂ *Attus superciliosus* Hentz.

*Dendryphantes elegans* Peckham.

Female 6$^{mm}$ long. Cephalothorax two-thirds as wide as long, and abdomen half as wide as long. The sides of the cephalothorax are

nearly straight and parallel in the female, and widened a little behind the middle in the male.

The colors of the living female, Pl. XVIII, fig. 3, are bronze green, which in some lights changes to copper red on the abdomen and cephalothorax. The legs are yellow, with longitudinal dark stripes, except the front femora, which are dark brown. The palpi are bright yellow at the end and dark at the base. In alcohol the colors appear dull and darker.

The males, fig. 3*d*, are much more brightly colored. The legs are orange, darker toward the ends, with fine dark, longitudinal stripes. The ends of the front tibiæ are dark brown, and have long, brown hairs on the inner and under side. The palpi are orange, darker toward the end. The sides and hinder part of the cephalothorax are orange, and there is a white line each side over the coxæ. The upper part of the cephalothorax and abdomen are covered with greenish yellow scales. On the front of the head are tufts of long hairs, yellow, mixed with black, pointing forward and a little inward between the middle and lateral eyes, fig. 3*e*. On the hinder end of the abdomen is an iridescent purple spot. The abdomen is green on the under side, and the sternum and coxæ are orange. In alcohol the colors become dull yellow and brown.

The mandibles of this species are slender, and the claw short, and strongly curved inward toward the point. In the male the mandibles are a little longer, and have a slight notch on the inner side, one-third their length from the end. Fig. 3*e*.

The epigynum is wide, with two anterior openings more than their diameter apart, and the posterior notch is divided into two. Fig. 3*h*.

The male palpi have the patella as wide as long, and the tibia still shorter, with a very short and small hook on the outer side. Fig. 3*g*. The tube of the palpal organ is twisted at the end, and has a spherical base, partly covered by the softer part of the bulb. Fig. 3*f*. The male has the legs of the first pair much larger than the others, and all the legs longer than the female. In the female the fourth legs are longest.

Some males have the colors darker and the front legs less elongated than usual, and do not have the black spot and black tuft of longer hairs on the ends of the tibiæ. They also have the tufts of hair over the eyes much smaller. Figs. 4, 4*a*. The palpal organs are a little stouter. Figs. 4*b*, 4*c*. Specimens from the Adirondacks and White Mountains are of this variety.

White Mountains, N. H., to New Haven, Conn.

**Icius hartii**, new sp.

The name *Icius Hartii* was given this species by Mr. Peckham, but not published. A specimen in the Museum of Comparative Zoology in Cambridge, is named by Keyserling *Icius tibialis* Koch.

Of this species I have only one female from Medford, Mass. This is $7^{mm}$ long, cephalothorax $2^{mm}$ long, and $1^{mm}$ wide. The cephalothorax is straight at the sides, very little narrowed behind. The abdomen is oval, a little pointed behind. Pl. XVIII, fig. 5. The length of the legs is 4, 1, 2, 3. The front legs are thickest, and the tibia is more than twice as thick as that of the other legs. In the male, fig. 5*a*, the front legs are longest and still more thickened than in the female. The cephalothorax is dark brown, with gray hairs. The abdomen has the middle dark and the border white ; the middle area broken at the edges by three or four pairs of white spots. The under side of the abdomen is light in the middle and black around the edges up to the white border of the upper surface. The femora are dark, except the third pair, which has light stripes. The other joints are dark at the ends, and have dark longitudinal stripes. The epigynum is small, and has two small openings directed forward about their diameter apart. Fig. 5*d*.

Some of the Wisconsin specimens which I have from Mr. Peckham, are a little larger, and the dorsal markings are more broken by oblique rows of white spots. The males have the abdomen shorter and the front legs much longer and thicker. The mandibles are a little longer than in the females. The male palpi are short and stout, the patella and tibia are as short as wide, and the tibial hook is very small and pointed, and directed downward. Fig. 5*c*.

The palpal organ is long enough to nearly cover the tibia. The tube is short and stout, and a little swelled at the base. Fig. 5*b*.

**Icius formicarius**, n. sp.

Length $5^{mm}$. Cephalothorax and abdomen usually about the same length. The general appearance is ant-like. The color is black with dark bronze green scales, except a white line each side of the thorax, and white stripes on the legs, two above and one beneath. One specimen has a few white scales on the spinnerets.

The cephalothorax is two-thirds as wide as long and the sides are nearly straight and parallel. The abdomen is widest across the hinder half and a little pointed behind. Both cephalothorax and abdomen are less flattened than in the other species. The legs are

long and slender, the fourth pair longest and the first next. The first pair are only a little stouter than the others. Pl. XVII, fig. 6. The epigynum is large with the anterior openings wide and far apart. The notch behind is very deep and narrow. Fig. 6*b*.

I have seen females only from Salem and Medford, Mass., and New Haven, Conn.

### Mævia vittata.

*Astia vittata* Peckham, *Attus vittatus* Hentz, *Attus niger* Hentz. ♂ *Mævia pencillata* Koch.

This is a large and brightly colored spider with long legs. The female is 8$^{mm}$ long, the male smaller but with the legs longer. Pl. XIX, figs. 1, 1*a*, 1*b*.

The living female has the legs and palpi translucent, a little yellow or greenish white. They are marked with indistinct light gray rings and black spots at the base of the hairs and spines. The cephalothorax is dark brown between the eyes and translucent like the legs in the thoracic part. There is a fine black line in the middle and on each side and a few gray marks radiating from the dorsal groove. The whole top of the cephalothorax is covered with greenish yellow scales mixed with gray hairs. The eyes are black and one female has a red stripe under the eyes each side. The abdomen is covered with scales which in the middle and at the sides are gray and mixed with black hairs. There are two longitudinal bands of light copper red along the back of the abdomen and indistinct angular marks of the same color in the middle of the hinder half. The colors of the under side are light gray and yellow spotted with gray on the abdomen.

According to Peckham, males of two very different colors belong to this species. One kind resembles the female. Fig. 1*b*. The red bands on the abdomen are broken up into rows of spots connected with the middle angular markings. The gray and black spots on the legs and cephalothorax are larger and there are several black marks on the front of the abdomen.

The palpi are bright orange yellow with the tibial hook black and a black spot on the inner side of each joint. The size of the black spot varies in different individuals and so passes into the other variety in which the cephalothorax and abdomen are entirely black and the palpi black except a few orange hairs on the outer side. The black cephalothorax and abdomen are covered with dark greenish shiny scales. The legs in this variety are white except the hairs. On the

front of the head of the black variety are three tufts of long hairs which are entirely wanting in lighter colored males. Fig. 1*a*. Although males of both kinds pair with the same female their behavior when mating is different, for an account of which see Peckham's article in Occasional Papers of the Nat. Hist. Soc. of Wisconsin, vol. i, 1888.

The cephalothorax of the female is widest behind the middle and is there two thirds as wide as long. It is narrowed to half that width at the hinder end and slightly narrowed toward the front, the head being not much more than half as wide as the cephalothorax is long. Fig. 1. The top of the cephalothorax is flat, rising a little between the dorsal eyes and the front row of eyes is straight on the upper edge. The comparative length of the legs is 4, 1, 3, 2 and the fourth pair is as long as the body.

The male palpi are long. The tibial hook is straight and blunt and about as long as the tibia itself. The tarsus is short and wide and turned outward at the tip. The palpal organ is oval with a long tube bent around the end of the bulb with its point in the groove on the outer corner of the tarsus. Fig. 1*c*.

The epigynum has a small oval opening very far farward and a slight notch on the edge of the transverse fold. Fig. 1*d*.

This is an active species, living on plants in summer.

Eastern Massachusetts and New Haven and Meriden, Connecticut.

**Marptusa familiaris** Peckham.

*Attus familiaris* Hentz.

The female is $10^{mm}$ long and the male nearly as large. The fourth pair of legs is longest in the female and the first pair in the male. The cephalothorax and abdomen are both much flattened. The cephalothorax is rounded at the sides and nearly twice as wide in the middle as in front. The abdomen is twice as long as wide, widest in the middle and truncated at the front end and sometimes at the hinder end also. In the latter case the spinnerets are so far under the abdomen that they cannot be seen from above. The legs are long and stout, the fourth pair one and a half times as long as the abdomen.

The general color is gray, covered with long gray and white hairs. The cephalothorax has a dark brown band along the edge each side which is larger and darker in the males. The abdomen has in the middle a yellowish white marking, covering half its width, the front half straight and the hinder half notched at the sides. Pl. XIX, fig.

3. The legs are darker at the ends of the joints and lighter in the middle. The under side of the abdomen is light at the sides and has a dark stripe in the middle.

The male palpus has the tibia very short and the tibial hook is long and slender and slightly thickened at the tip. The tube of the palpal organ starts from the middle of the inner side of the bulb and turns obliquely across the end of the tarsus. Near the end of the tube but not quite reaching the tip is a short flat process. Figs. 3*b*, 3*c*.

**Epiblemum scenicum** Thorell. *Epiblemum faustum* Hentz. *Salticus scenicus* Latr. *Callielhera scenica* Simon. *Epiblemum scenicum* Peckham.

Gray with white markings. On some the white marks are much more definite than on others, the gray ground having very few white scales mixed with it. I have usually found the spiders of this variety about houses, while those from the country, living on plants, have white as well as yellow scales largely mixed with the gray so as to obscure the white markings. The field specimens are usually more slender.

The front of the head around and above the eyes is white. There is a definite white stripe on each side of the cephalothorax and in the middle of the cephalothorax two white spots one on each side of the dorsal groove. On the abdomen there is a white stripe across the anterior end and two oblique marks on each side. Pl. XIX, fig. 2.

The legs are gray with white rings not very distinctly marked and the palpi white. The markings are very different from those of any other species.

The proportions of the body differ considerably in individuals, some appearing much longer and more slender than others. The cephalothorax is about two-thirds as wide as long and the abdomen usually about the same width and longer.

The epigynum is raised at the hinder edge, a little pointed and with a round notch about as deep as wide. The anterior half is a little narrowed and notched in the middle with a depression on each side, in the bottom of which is a round hole. Fig. 2*e*. The shape of the epigynum varies and in pale or freshly moulted females the internal parts may be seen through the skin as shown in Peckham's figure.

The males differ but little in color and markings from the females, but the palpi are large and the mandibles two-thirds as long as the cephalothorax and turned forward almost horizontally. Fig. 2*a*, 2*c*.

The tibia of the male palpus is shorter than the patella and at the distal end as wide as long, with a stout pointed hook directed forward and a little inward. Fig. 2*f*.

Found occasionally under stones or on bushes but common on the outside of houses and fences in the warmest and driest places. The colors resemble closely that of unpainted wood stained by the weather.

Common all over New England. A common European species.

**Menemerus lineatus.** *Icius lineatus* Peckham. *Altus quadrilineatus* Peckham, 1883.

A small spider only $4^{mm}$ long and very distinctly marked with two white longitudinal lines on the middle of the abdomen and two others on the sides so far down that only the front ends of them can be seen from above. Pl. XIX, fig. 5.

The color in life is dark brown. The white lines on the back have their edges very dark. The cephalothorax and dark parts of the abdomen are thinly covered with long, slender, yellow scales mixed with a few white hairs on the front of the abdomen. On the under side of the abdomen are four white lines. The legs are dark brown, the first pair darkest.

The relative length of the legs is 1, 4, 2, 3. The first pair are twice as thick as the others. The top of the cephalothorax is flat two-thirds its length from the front. There is a short, transverse groove a little behind the dorsal eyes. The cephalothorax is widest across the middle but only very little wider than in front. The epigynum has a dark ridge behind, curved at the ends around two round holes. Fig. 5*b*.

Manchester and Medford, Mass.

**Menemerus binus.** *Attus binus* Hentz, not *M. paykullii* Peckham, 1885.

Female $9^{mm}$ long. Legs 1, 4, 2, 3, The cephalothorax is widest behind the middle and narrows slightly toward the front of the head where it is half as wide as long. The cephalothorax is flat above and a little swelled around the dorsal eyes. There is a short transverse groove behind the eyes. Pl. XIX, fig. 4.

The abdomen is half as wide as long, narrowed at both ends. The first pair of legs are about as long as the fourth pair, but twice as thick. The second pair of legs are slightly thicker than the third and fourth.

I have not seen the colors in life. In alcohol the cephalothorax is dark brown except around the edges where it is yellowish. The front half of the cephalothorax between the eyes is dark purple when turned to the light. The abdomen is white with two longitudinal black stripes that do not reach either end. The front legs are orange color with light brown stripes and a black spot near the end of each joint on the inner side. The other legs are dirty yellow with indistinct brownish stripes at the sides. Under the abdomen is a black middle stripe.

The epigynum has the openings very small and wide apart and the posterior notch very wide with square corners at the ends. Fig. 4*a*.

One female from Meriden, Conn. Mr. Peckham has the same species from Nebraska.

**Neon nellii** Peckham.

This is the smallest of our New England Attidæ, being only 2.5 to $3^{mm}$ long with the cephalothorax not much over $1^{mm}$. The general color is dark gray, darkest toward the head, so that the spider is hard to see on gray stones or weathered wood. The cephalothorax is high, the highest part being a little behind the middle, from which it curves downward to the front eyes and slopes more abruptly backward. The eyes are large and prominent, the first row nearly straight and as wide as the widest part of the cephalothorax. The posterior eyes are nearly as large as the front middle pair and are at the middle of the cephalothorax. The abdomen is a little pointed behind and the spinnerets are large for the size of the spider. Pl. XX, fig. 1.

The cephalothorax is smoky gray, darker toward the front and darker in the males than females. The abdomen is gray with yellowish white markings in a herring-bone pattern through the middle. The underside of the body is light gray or whitish. The legs are gray, darker toward the front.

The epigynum is large and has two rounded openings toward the front. Fig. 1*b*. The male palpi are also large and the palpal organ extends backward so as to cover the short tibia. The tube lies obliquely across the end of the bulb, its tip resting in a groove on the end of the tarsus. Fig. 1*c*.

A common species under stones and leaves at all seasons.

**Euophrys monadnock**, new sp.

One male was found on the upper part of Mt. Monadnock, N. H., on the rocks. Pl. XX, fig. 2. It is $4^{mm}$ long, the cephalothorax and abdomen about the same length. The legs are all long, relative length 4, 1, 3, 2. The ends of the palpi are white as far as the end of the femur. The tarsi of the first and second legs, and the tips of the tarsi of the third and fourth are white. The femora of the third and fourth legs are white, in life, light pink. The rest of the body is deep black. The first and second legs are a little thickened and the metatarsus, tibia and patella, and the end of first femur covered with long scales, as long as the diameter of the leg. The first and second legs are bright purple toward a bright light.

The front of the head is wide, and the sides of the cephalothorax nearly straight and parallel. Fig. 2*c*. The front of the head, below the eyes, slopes inward, and the sternum is short and wide. Fig. 2*a*.

The palpi are long and slender, the tarsus not much wider than the other joints. The tibial hook is straight and very slender, not much thicker than one of the hairs. Fig. 2*d*. The palpal organ is long and narrow at the base, where it extends over the tibia. The tube is short and curved around the end of the bulb. Fig. 2*e*.

A female, which probably belongs to this species, was found in Dublin, N. H., three miles from Monadnock. It is $5^{mm}$ long, with short and thin hairs and colors like *Tegenaria*. The cephalothorax has the sides straight and parallel, three-fourths its length from the front, and is a little rounded behind. Fig. 2*f*. The eyes are large and the lateral are more than half as large as the front pair. The posterior eyes are as far apart as the length of the front row, and little more than half as far from the lateral. The abdomen is oval, widest behind the middle. The legs are 4, 1, 2, 3, the front pair much thickened and the second slightly so. The cephalothorax is dark brown in front and lighter toward the back, marked with dark radiating lines. The abdomen is gray, with light, angular markings along the middle, and irregular oblique lines at the sides. The legs are without markings, the first pair darkest. The epigynum has two large openings about their diameter from the transverse fold. Fig. 2*g*.

**Euophrys cruciatus**, new sp.

This species is more generally covered with scales than *monadnock*, the front tibiæ are less thickened, and do not have the long

scales of that species, and the colors are less bright and more like those of *Attus palustris.* The only specimen I have, a male, is $5^{mm}$ long. Pl. XX, fig. 8. The cephalothorax is three-fourths as wide as long, widest behind the middle. It is low in front, and the front row of eyes are turned a little downward, the lateral pair half their diameter, higher and farther back. The middle eyes are nearer the dorsal than lateral. The dorsal eyes are nearer together than the lateral pair. The abdomen is as long and as wide as the cephalothorax, widest in the middle and pointed behind.

The lengths of the legs are 4, 1, 3, 2, the fourth pair very long. The first and second pairs are a little thickened.

The cephalothorax of my specimen is so much rubbed that the markings are nearly destroyed; it shows only long black hairs over the eyes, and irregular white markings behind the eyes and at the sides. The abdomen is black, with a distinct white cross in the middle. Fig. 8. The legs have dark and light rings, which were probably covered with white and black hairs. The tibia of the palpi is light, and the other joints dark, with long hairs.

The tibia of the male palpus is wider than long. The tibial hook is nearly as long as the tarsus, and curved to fit along its edge. The tarsus is strongly curved downward. The palpal organ is oval, and the tube begins near the outer end, curves around its base and again outward through a groove in the edge of the tarsus to a notch near the end of the tibial hook. Figs. 8*b*, 8*c*, 8*d*.

Dublin, N. H., July 16.

**Phlegra leopardus.** *Attus leopardus* Hentz.

Very distinctly marked with two white lines on the cephalothorax, as far up as the dorsal eyes, and three white lines on the abdomen. The female is $8^{mm}$ long, the cephalothorax $3.5^{mm}$. The cepholothorax is two-thirds as wide as long and a little widest behind the middle. The lateral front eyes are high enough for their middle to be opposite the top of the middle eyes. The dorsal eyes are unusually far forward; they are as far apart as the front lateral eyes, and two-thirds as far from the front of the head. Pl. XXI, fig. 1. The cephalothorax is rounded upward from the front to the dorsal eyes, and from there backward is flat more than half-way to the hinder end. The abdomen is widest in the middle and a little pointed behind. The legs are short, and their relative length 4, 3, 1, 2. The first and second pairs are thicker than the other. The cephalothorax is dark brown, darkest between the eyes. A white stripe extends

from the front of the head, over the eyes each side the whole length of the cephalothorax. A middle white stripe begins between the middle eyes, but fades out between the dorsal eyes. The legs are light brown, with two darker rings on each joint. The abdomen is brown, with a middle and two lateral white stripes. The abdomen is light on the under side and the sternum, legs and mouth parts dark brown. The epigynum has two large round openings near the hinder edge, and a small, square-cornered notch in the edge. Fig. 1*b*.

The male is darker than the female, but marked in the same way on the cephalothorax and abdomen. The white bands on the cephalothorax are mixed with red between the eyes. The legs are longer than those of the female, and not marked with rings.

The male palpus has the tibia short and wide on the upper side, with a deep notch on the outer side, and a short hook. Figs. 1*c*, 1*e*. The bulb of the palpal organ extends backward on the outer side so as to cover the tibia. Fig. 1*d*.

Female from Mt. Tom, Massachusetts. Male from Blue Hill, Milton, Mass. Another female was seen in Medford, Mass.

**Hasarius Hoyi**, Peckham.

Female 6 or $7^{mm}$ long. The cephalothorax is shorter and the abdomen larger than in the nearest species. Pl. XXI, fig. 2, 2*a*. The cephalothorax of the female is three-fourths as wide as long, and a little enlarged in the middle. The posterior eyes are two-thirds as far from the front row as from each other. Legs 1, 4, 2, 3, all nearly of the same length. First and second pairs a little thickened.

The markings vary, but in alcohol there is always a light marking across the back of the cephalothorax, a little behind the dorsal eyes, and turning forward on each side as far as the front eyes. In front and behind this marking the cephalothorax is dark brown. On the abdomen there are light and dark oblique markings forming, especially in young individuals, a regular light herring-bone marking in the middle.

In life, the light markings are partly white and partly orange-brown. A brightly marked young male has the cephalothorax white in front, both above and below the eyes. Fig. 2. A white band on each side turning upward and inward behind the dorsal eyes, bnt not united in the middle. Hinder part of cephalothorax below and between the white bands black. There are two black bands behind the dorsal eyes, extending obliquely through the dorsal groove to the white

bands at the sides. The rest of the top of the cephalothorax is covered with orange scales.

Abdomen with a white band around the front and sides. Middle of the back black, with a few orange scales around the edges. Sides of the black patch irregularly scalloped. In the middle are oblique white markings, edged with orange-brown.

The legs are white at the ends and dark on the patella and tibia, and outer end of the femur. Ends of all the joints darker than the middle.

The epigynum has two large anterior openings near together, and behind and each side of them two other large openings near the edge. Fig. 2*e*.

The adult male has the colors much like the young male, described above, with the legs black on the patella and tibia, and part of the femur, and the rest white, with black at the ends of the tarsus and metatarsus. The female is various shades of brown, mixed with white and yellow scales and black hairs. Fig. 2*b*, 2*c*.

The male palpi are short, black at the base and white on the top of the tarsus. The tibial hook is long and blunt, extending along the side of the tarsus half its length. Fig. 2*f*, 2*g*. The palpal organ has the inner posterior corner swelled and extended inward to a blunt point. Fig. 2*f*. The tube curves around from the base to the tip of the tarsus.

A common species in eastern Massachusetts. Found also from the White Mountains to southern Connecticut.

**Habrocestum splendens**, Peckham. ♂ *Ergane splendens* Keys.

♀ *Pellenes nigriceps* Keys.

I have only seen the males of this species. It has been fully described by Peckham in his N. A. Attidæ in the Transactions of the Wisconsin Academy, vol. vii, and figured and its mating habits described in vol. i, of Occasional Papers of the Nat. Hist. Society of Wisconsin, Milwaukee, 1889.

My males are $5^{mm}$ long, with the cephalothorax nearly $3^{mm}$. The cephalothorax is widest and highest behind the second legs. The front part projects forward beyond the mandibles and the front eyes look a little downward. Pl. XX, figs. 5, 5*b*. The abdomen is shorter than the cephalothorax, widest in the middle, square in front and pointed behind. The legs are long and stout, the third pair longest. In alcohol the brilliant colors of the male fade, and the colors and markings resemble more those of the female. The cephalothorax is

dark brown, with a transverse light marking behind the eyes. The whole cephalothorax is thickly covered with light scales, and the front half with black hairs between them. The legs and palpi are light at the base and darker brown toward the tips, covered with scales and hairs, either black or various shades of gray. The abdomen is marked with three or four pairs of dark spots, united together more or less in different spiders, the front pair usually forming a single transverse mark, with a light mark in front of it. The light portions of the abdomen are yellowish in the middle, and bright copper-red at the sides.

The male palpi have the femur long, the patella about as long as wide, and the tibia half as long as wide. The tibial hook is large and flat, fig. 5*d*, and lies against the tarsus, nearly half its length. The tarsus is short and wide, and the bulb of the palpal organ flat and circular. The tube starts at the base and curves around the inner side, ending in a short groove, pointing outward on the end of the tarsus. Fig. 5*c*.

Beverly, Mass. Clarksville, near Albany, N. Y. Young in winter in old cocoons of *Argiope*, on marshes between Boston and Brookline, Mass.

**Habrocestum peregrinum,** Peckham.

These specimens have been identified for me by Mr. Peckham.

The female is $6^{mm}$ long, the male a little smaller ; both sexes smaller than *H. splendens.* The area between the eyes slopes forward and is covered with short scales mixed with longer hairs, light brown in front and darker behind, where it meets a white marking pointed forward in the middle and curved backward and outward behind the eyes. Pl. XX, fig. 6. On each side of the cephalothorax is a white stripe, extending from the front lateral eyes backward under the eyes, and then curved upward and again downward to the end of the thorax. The abdomen has three white stripes, with black between, narrower and sharper in the male. The under side of the body and legs are light brown, with white and black hairs.

The legs of the third pair are very long in both sexes, and in the male there is a peculiar shape to the patella, fig. 6*b*, which is flattened and widened at the distal end, where there is a stout spine extending over the tibia. On the front of this flattened patella is a black spot. Mr. Peckam gives an account of the use of this ornamental patella in the pairing of an allied species in " Occasional Papers of the Nat. Hist. Soc. of Wisconsin, vol. i, No. 3, 1890." Peck-

ham's description says, two short spines on tibia of first pair, but my male has three pairs of spines on tibia and two pairs on metatarsus.

The male palpus differs little from that of *splendens*. The tibial hook is a little sharper, and the angle of the tarsus just over it, a little more prominent.

The epigynum has a simple round opening directed forward, from which a tube extends backward. Fig. 6*a*.

I have only three specimens—an adult male and female, and one immature male from Hyde Park, Mass. Mr. Peckham has it from New York and Connecticut.

**Saitis pulex**, Peckham.

*Attus pulex* Hentz. *Saitis X-notata* Keyserling. *Cyrba pulex* Keyserling.

A common spider 4 or 5$^{mm}$ long, the female gray or brown of different shades, and resembling gray stone or dried leaves, on which it lives. The cephalothorax and abdomen are of the same length, the abdomen of the female wider than the cephalothorax, widest across the middle and pointed behind. Pl. XX, fig. 7*a*. The cephalothorax is half longer than wide, widest behind the middle. The eye-space is half wider than long, narrower behind than in front. The third and fourth pairs of legs are of the same length, and longer than the first and second. The cephalothorax has a light stripe in the middle, between the eyes, that narrows backward to a point at the hinder end. The abdomen has two nearly parallel light lines in the middle of the front half and behind them a transverse marking pointed at the sides, behind and around which are small, irregular light marks. The legs are covered with alternate dark and light spots.

The epigynum has two large openings near together, and near its hinder edge. Fig. 7*g*.

In the male the abdomen is smaller and narrower, the head higher and the colors brighter. Fig. 7. The cephalothorax, between the eyes and a little behind them, is black. The front and sides of the head below the eyes, and the hinder half of the cephalothorax are yellow, or orange. The abdomen is black with markings like the female. The legs have the tarsi yellow and the other joints indistinctly marked with longitudinal yellow lines. The palpi are yellow, except the ends of the tarsi which are black. On the under side the legs and abdomen are black. The coxæ are yellow, with a black line in the middle and the sternum, maxillæ and mandibles are yellow; the sternum sometimes with a black middle line. The male palpi

have the tibia very short, with a flat, thin hook. Fig. 7*e*, 7*d*. The tube of the palpal organ is short and curved in a circle on the end of the bulb so that the tip points inward. Fig. 7*c*.

Massachusetts and Connecticut.

**Attus palustris**, Peckham.

Female 6$^{mm}$ long, male 5$^{mm}$. The cephalothorax is three-fourths as wide as long. The abdomen is short, a little wider than the cephalothorax and pointed behind. Pl. XX, fig. 3. The cephalothorax is light brown in the female and dark in the male, with a narrow, white stripe in the middle, widened between the dorsal eyes, and a white stripe each side, as high as the dorsal eyes. The abdomen has a wide, white transverse marking, just behind the middle, and several angular marks behind it. On the front half are two white spots. In the male the large middle marking is usually divided into two white spots.

The relative length of the legs is 4, 1, 2, 3 in the female, and 1, 4, 2, 3 in the male. The dorsal eyes are very far forward, little more than half as far from the front eyes as they are from each other.

The epigynum has a large oval opening divided in front into two. The hinder edge over the transverse fold has a small projection in the middle. Fig. 3*c*.

The hook of the tibia of the male palpus is nearly straight and has a short piece at the tip very narrow. Fig. 3*b*. The palpal organ is oval, and the tube extends from the base around the inner side, the end pointing outward. Fig. 3*a*.

Portland, Me.; Eastern Mass.; New Haven, Conn.

**Attus sylvestris**, new sp.

Male 4$^{mm}$ long. Cephalothorax almost as wide as long. Abdomen smaller than cephalothorax; legs shorter and stouter than in *A. palustris*, and the male palpi nearly as large as in the male *palustris*, which is half larger. The colors resemble those of *Saitis pulex*. The legs are distinctly ringed with dark and light brown. The cephalothorax is dark brown, covered with lighter hairs, which are nearly rubbed off in the specimens. The male palpi have the tarsus dark brown, and the rest of the palpus lighter above but dark beneath. The tibia and patella are covered with white hairs that are very long on the sides of the tibia. The palpal organ resembles closely that of *A. palustris*, and is nearly as large. The markings

of the abdomen are dark and light brown, and resemble those of *Saitis pulex* and the female *A. palustris*. There is a large light mark across the middle of the abdomen, and behind it several light angular marks. In colors and general appearance this resembles *Saitis pulex*, and lives in the same places under stones and leaves.

Beverly and Middleton, Massachusetts.

**Synemosyna formica**, Hentz.

This is the most common ant-like spider, and the most ant-like among them. It is $6^{mm}$ long, and the cephalothorax is $2^{mm}$ long and $1^{mm}$ wide. The cephalothorax is narrowed behind and the abdomen in front and each has a deep depression near the middle. Pl. XXI, fig. 5. The pedicel of the abdomen is flat and widened behind, so that it is nearly as wide as the ends of the thorax and abdomen which it connects. The front middle eyes are large, and cover two-thirds of the front of the head. The rest of the eyes are very small. The legs are slender, their relative length 4, 3, 1, 2. The general color is black, with yellowish white markings. There is a triangular white spot in front of the dorsal groove, and one on each side widening downward, under the posterior eyes. On the abdomen there is a white stripe extending downward from the dorsal groove on each side, and uniting in a large white patch on the front of the under side of the abdomen. The second legs are entirely white, the first have a black stripe along the inner side, the third have the femur black and a black spot at the base of the tibia, and the fourth have the femur, the end of the patella and nearly all the tibia black, and black spots on the front of the coxæ and trochanter, and black lines on the metatarsus. The palpi are white, sometimes with dark spots on the sides of the joints.

The front end of the abdomen has a hard piece on the under side from the pedicel to the epigynum, which extends upward on each side, so that the edges are visible from above. The epigynum has a wide oval opening, in the front part of which are two small openings to the tubes.

The males differ but little from the females in color and general appearance. The head is higher and narrower, and the mandibles a little longer, turned obliquely forward and curved apart in the middle. Fig. 5*c*. The male palpi are large. The tibia is short and has a very large hook. Fig. 5*f*. The palpal organ is large and only half covered by the tarsus. The tube passes completely around

the bulb, then passes through the usual groove to the outside of the tarsus, where it is coiled once around the flattened end. Fig. 5*e*.

This species lives on plants and matures early in the summer. At first sight it resembles an ant, but its gait is slower and steadier. I have never seen it jump but it runs quickly when frightened. It lives in summer on bushes, and matures in June. In the latter part of summer only young are to be found, and in the winter it hides under leaves on the ground.

Common at New Haven, Conn., and around Boston, Mass.

**Salticus ephippiatus**, Peckham.

*Synemosyna ephippiata* Hentz.

This spider is $7^{mm}$ long, the cephalothorax $3^{mm}$ long, and half as wide. In color and general appearance it resembles orange-brown ants. The cephalothorax is high in front and low behind the dorsal groove, which is in a slight depression. Pl. XXI, fig. 4*a*. The sides of the cephalothorax are nearly straight for two-thirds its length, and it is only slightly narrowed behind. The abdomen is oval, a little narrower in front than behind. The legs are long and slender, their length 4, 1, 3, 2. Fig. 4.

The colors are various shades of orange-brown, some individuals being very dark, and others pale. There is an indistinct light mark across the middle of the cephalothorax and across the abdomen, a little in front of the middle, and sloping backward down the sides, nearly to the spinnerets.

The femora of all the legs are dark, except a light stripe on the front of those of the first pair. The patella and tibiæ are all light on the upper side and darker beneath. The metatarsi of the hind legs are dark, the others partly light in the middle, or on the upper side. The tarsi of the front legs are dark, the others light. The coxæ of the third legs are dark, the others light. The palpi of the female have the tibia and tarsus very much thickened and covered with stiff hairs on the under side. The palpi are colored as dark as the head. The epigynum has two small openings near together, and turned toward each other. Fig. 4*c*.

Eastern Massachusetts and New Haven, Conn., under leaves, and on plants in summer.

**Synageles picata,** Peckham.

*Synemosyna picata* Hentz.

I have one young male from New Haven, Conn., that appears to be of this species. Pl. XXI, fig. 3. It is as large as adults sent me from Wisconsin by Mr. Peckham, but not as distinctly marked. The cephalothorax has the two white spots in the middle, but is not depressed at this part as in the adult. Fig. 3*a*. The coloring is more uniform than in the Wisconsin specimens. The white marks on the abdomen show indistinctly as do the markings of the legs. The specimen is $3.5^{mm}$ long.

The adults of both sexes have a slight elevation around the posterior eyes, and a distinct depression across the cephalothorax, just behind the eyes. Fig. 3*b*. The front legs of the female are twice as thick as the other legs, fig. 3*c*, and the front legs of the male are still thicker, especially in the femur and tibia. Fig. 3*d*. The patella and tibia are flattened in front, more in the male than in the female. The front of these legs, and the front of the cephalothorax of the male, are purple and iridescent in a bright light. In alcohol they become dark brown. The abdomen has two white stripes extending down the sides in the shallow depression across the front third. The abdomen in front of the depression is rounded, and sometimes swells in alcohol to an unnatural width. The hinder half of the cephalothorax and front of the abdomen are lighter colored than the parts before and behind.

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## EXPLANATION OF PLATES.

### Plate XVI.

Fig. 1. *Phidippus rufus*, female × 4; 1*a*, male × 4; 1*b*, female, nearly full grown; 1*c*, young; 1*d*, young soon after leaving the cocoon; 1*e*, epigynum; 1*f*, epigynum of another female; 1*g*, immature epigynum; 1*h*, male palpus; 1*i*, male palpus; 1*j*, brown scales from young female; 1*k*, yellow scale from young female.

Fig. 2. *Phidippus brunneus*, epigynum.

Fig. 3. *Phidippus mystaceus*, female × 4; 3*a*, 3*b*, male palpus; 3*c*, epigynum; 3*d*, posterior notch of epigynum of another female.

Fig. 4. *Phidippus ruber* Keys; male × 4; 4*a*, epigynum; 4*b*, male palpus; 4*c*, male palpus.

Fig 5. *Phidippus tripunctatus*, female × 4; 5*a*, end of mandibles of male; 5*b*, male palpus; 5*c*, patella and tibia of male palpus; 5*d*, white scales from middle of abdomen.

PLATE XVII.

Fig. 1. *Dendryphantes militaris*, female × 4; 1*a*, male × 4: 1*b*, mandible of male, under side; 1*c*, mandible of male, upper side; 1*d*, male palpus.

Fig. 2. *Dendryphantes æstivalis*, female of light variety × 4; 2*a*, cephalothorax of same female after wetting with alcohol; 2*b*, female of small dark variety; 2*c*, small male × 8; 2*d*, 2*e*, male palpus; 2*f*, epigynum.

Fig. 3. *Dendryphantes montanus*, cephalothorax of female; 3*a*, cephalothorax of male; 3*b*, mandible of male; 3*c*, epigynum with two forms of posterior notch; 3*d*, 3*e*, male palpi.

Fig. 4. *Zygoballus bettini*, female × 6; 4*a*, mandibles of male under side; 4*b*, male palpus.

Fig. 5. *Zygoballus terrestris*, female × 8; 5*a*, mandible of male; 5*b*, epigynum; 5*c*, male palpus; 5*d*, outer side of tibia of male palpus.

PLATE XVIII.

Fig. 1. *Icius palmarum*, female × 4; 1*a*, side of cephalothorax of female; 1*b*, front of head of a male with short mandibles; 1*c*, cephalothorax of male with long mandibles; 1*d*, under side of mandibles of male; 1*e*, epigynum; 1*f*, another pattern of dorsal markings of female; 1*g*, male × 4; 1*h*, male palpus; 1*i*, male palpus, showing tibial hook.

Fig. 2. *Icius mitratus*, male × 4.

Fig. 3. *Icius elegans*, female × 4; 3*a*, side of cephalothorax; 3*b*, front of head of female; 3*c*, under side of cephalothorax; 3*d*, male × 4; 3*e*, front of head of male; 3*f*, 3*g*, male palpus, 3*h*, epigynum.

Fig. 4. *Icius elegans*, dark variety of male × 4; 4*a*, front of head; 4*b*, 4*c*, male palpus.

Fig. 5. *Icius hartii*, female × 4; 5*a*, male from a Wisconsin specimen from Mr. Peckham; 5*b*, male palpus; 5*c*, tibial hook; 5*d*, epigynum.

Fig. 6. *Icius formicarius*, female × 4; 6*a*, side of cephalothorax; 6*b*, epigynum.

PLATE XIX.

Fig. 1. *Mævia vittata*, back of female × 4; 1*a*, dark variety of male × 4; 1*b*, light variety of male × 4; 1*c*, male palpus; 1*d*, epigynum.

Fig. 2. *Salticus scenicus*, female × 4; 2*a*, male; 2*b*, side of cephalothorax of female; 2*c*, mandibles of male; 2*d*, male palpus; 2*e*, epigynum; 2*f*, tibial hook from above; 2*g*, tibial hook from side.

Fig. 3. *Marptusa familiaris*, back of female × 4: 3*a*, front of head and mandibles; 3*b*, male palpus; 3*c*, side of male palpus without tarsus; 3*d*, side of cephalothorax of female; 3*e*, 3*f*, epigynum of two individuals.

Fig. 4. *Menemerus binus*, female × 4; 4*a*, epigynum.

Fig. 5. *Menemerus lineatus*, female × 4; 5*a*, end of mandible; 5*b*, epigynum.

PLATE XX.

Fig. 1. *Neon nellii*, back of female; 1*a*, side of female; 1*b*, epigynum; 1*c*, male palpus.

Fig. 2. *Euophrys monadnock*, side of male; 2*a*, under side of cephalothorax of male; 2*b*, front of head; 2*c*, top of cephalothorax of male; 2*d*, 2*e*, male palpus; 2*f*, female; 2*g*, epigynum.

Fig. 3. *Attus palustris*, female × 4, legs in a natural position; 3*a*, male palpus; 3*b*, tibial hook from outside; 3*c*, epigynum.

Fig. 4. *Attus sylvestris*, male × 4.

Fig. 5. *Habrocestum splendens*, male × 6; 5*a*, front of head; 5*b*, side of cephalothorax; 5*c*, male palpus; 5*d*, tibial hook.

Fig. 6. *Habrocestrum peregrinum*, female × 4; 6*a*, epigynum; 6*b*, patella and tibia of third leg of male, front side.

Fig. 7. *Saitis pulex*, male × 4; 7*a*, female × 4; 7*b*, cephalothorax of male, side view; 7*c*, male palpus; 7*d*, 7*e*, tibial hook; 7*f*, face of male; 7*g*, epigynum.

Fig. 8. *Euophrys cruciata*, male × 4; 8*a*, front of head; 8*b*, male palpus from below; 8*c*, male palpus from side with tibial hook; 8*d*, end of the tarsus of male palpus; 8*e*, side of cephalothorax.

## Plate XXI.

Fig. 1. *Phlegra leopardus*, female × 4; 1*a*, under side of cephalothorax; 1*b*, epigynum; 1*c*. male palpus from above; 1*d*, male palpus, under side; 1*e*, tibial hook, outer side.

Fig. 2. *Hasarius hoyi*, young female × 4; 2*a*, same individual after keeping in alcohol; 2*b*, 2*c*, abdominal markings of adult females; 2*d*, dorsal markings of young female; 2*e*, epigynum; 2*f*, 2*g*, male palpus.

Fig. 3. *Synageles picata*, young female × 6; 3*a*, side of young female; 3*b*, side of cephalothorax of adult female from Wisconsin; 3*c*, first and second legs of female × 12; 3*d*, first and second legs of male × 12, from Wisconsin specimens.

Fig. 4 *Salticus ephippiatus*, female × 6; 4*a*, side of cephalothorax of female; 4*b*, palpus of female; 4*c*, epigynum.

Fig. 5. *Synemosyna formica*, female × 6; 5*a*, side of female; 5*b*, under side of mouth parts of male; 5*c*, front of head of male; 5*d*, under side of front of abdomen of male; 5*e*, palpus of male; 5*f*, tibial hook; 5*g*, epigynum.

## XV.—A Provisional List of the Hepaticæ of the Hawaiian Islands. By A. W. Evans.

During the years 1875 and 1876, Mr. D. D. Baldwin made a systematic collection of the Hepaticæ found growing in the Hawaiian Islands, particularly in the island of Maui. These were sent to Prof. Eaton for determination, and he, in turn, sent many of them to Mr. Austin, who published descriptions of several new species and returned to Prof. Eaton a list of his determinations, including many species previously described. The following list is based largely upon those determinations, but contains in addition several of Mr. Baldwin's species which Mr. Austin did not examine. All other species accredited to the Islands in the Synopsis Hepaticarum and in other publications are also mentioned in the list.

**Frullania arietina** Tayl. Syn. Hep. p. 413. Oahu (Mann and Brigham).

**F. squarrosa** Nees, Syn. Hep. p. 416. Oahu (Mann and Brigham); Hawaiian Islands (Hillebrand).

**F. Sandvicensis** Ångstr. Öfver. af. Kongl. Vet.-Akad. Förhandl. 1872, No. 4, p. 28. Hawaiian Islands (Andersson).

**F. hypoleuca** Nees, Syn. Hep. p. 443. Oahu (Meyen); West Maui (Baldwin, 81!).

**F. Kunzei** Lehm. et Lindenb. Syn. Hep. p. 449. Oahu (Mann and Brigham); West Maui (Baldwin, 191!).

**F. apiculata** Nees, Syn. Hep. p. 452. Oahu (Mann and Brigham); West Maui (Baldwin, 57! 165!).

**F. explicata** Mont. Syn. Hep. p. 452. Hawaiian Islands (Gaudichaud).

**Jubula piligera** (*Frullania Hutchinsiæ* Nees, var. β, Syn. Hep. p. 426; *F. piligera* Aust. Bull. Torr. Bot. Club, vi, p. 301). Hawaiian Islands (Baldwin, 147!).

**Lejeunea alcina** Ångstr. Öfver. af. Kongl. Vet.-Akad. Förhandl., 1872, No. 4, p. 23. Hawaiian Islands (Andersson).

**L. Sandvicensis** (*Phragmicoma Sandvicensis* Gottsche, Annal. des Sc. Nat. 4me série, T. viii, p. 344; *P. subsquarrossa* Aust. Proc. Acad. Nat. Sci. Phil., Dec. 1869). Hawaiian Islands (Gaudichaud, Andersson, Hillebrand); West Maui (Baldwin, 113!).

**L. elongata** Aust. (*Phragmicoma elongata* Aust. Proc. Acad. Nat. Sci. Phil, Dec. 1869). Hawiian Islands (Hillebrand, etc.); Kauai (Baldwin, 273!).

**L. Mannii** Aust. Bull. Torr. Bot. Club, v, p. 15. Oahu (Mann and Brigham!).

**L. gibbosa** Ångstr. l. c. p. 23. Hawaiian Islands (Andersson).

**L. Anderssonii** Ångstr. l. c. p. 24. Hawaiian Islands (Andersson).

**L. ungulata** Ångstr. l. c. p. 25. Hawaiian Islands (Andersson).

**L. Owaihiensis** Gottsche, Syn. Hep. p. 351. Hawaii (Herb. Hook.)

**L. oculata** Gottsche, Syn. Hep. p. 357. Hawaii (Herb. Hook.).

**L. stenoschiza** Ångstr. l. c. p. 26. Hawaiian Islands (Andersson).

**L. Pacifica** Mont. Syn. Hep. p. 378. Hawaiian Islands (Gaudichaud, Andersson),

**L. subligulata** (*L. cancellata* Lindenb. in Herb. non Nees et Mont.; *L. Sandvicensis* Steph. Hedwigia, xxix, p. 88). Hawaiian Islands (Gaudichaud).

**L. albicans** Nees, Syn. Hep. p. 386 (*L. cucullata* Lindenb. in Herb. non Nees; vid. Steph. l. c. p. 89). Oahu (Meyen).

**L. cucullata** Nees, Syn. Hep. p. 390. Hawaiian Islands (Andersson, Hillebrand).

**L. calyptrifolia** Dum.? (*L. calyptrata* Ångstr. l. c. p. 27). Hawaiian Islands (Andersson).

**L. Hillebrandii** Aust. Bot. Gazette, i, p. 35. Hawaiian Islands (Hillebrand).

**L. ceratocarpa** Ångstr. l. c. p. 27 (? *L. obcordata* Aust. Bot. Gazette, i, p. 36). Hawaiian Islands (Andersson); West Mauii (Baldwin).

**Radula reflexa** Nees et Mont. Syn. Hep. p. 253. Hawaiian Islands (Gaudichaud).

**R. Xalapensis** Mont. Syn. Hep. p. 255. Hawaiian Islands (Baldwin, 232!).

**R. Javanica** Gottsche, Syn. Hep. p. 257. Hawaii (Herb. Hook.); East Maui (Baldwin, 50, 179!).

**R. Mannii** Aust. Bull. Torr. Bot. Club, v, p. 15. Oahu (Mann and Brigham!); East Maui (Baldwin, 21!).

**Porella Hawaiiensis** n. sp. West Maui (Baldwin!).

Brownish-green, cæspitose; stems one- to three-pinnate; leaves loosely imbricated, spreading, falcate-ovate, the margins mostly entire or toothed towards the apex, which is coarsely and irregularly spinulose-dentate, the ventral margin distinctly folded at about the middle; lobule narrow, lingulate-ovate, with entire or undulate margins; amphigastria about the width of the stem, ovate, mostly entire, the apex variously one- to four-toothed; fruit not seen.

Leaves about $1{\cdot}5^{mm}$ long, $0{\cdot}5^{mm}$ wide; cells in middle of leaf $0{\cdot}025^{mm}$ in diameter.

Allied to *Madotheca ligulifera* Tayl. (Syn. Hep. p. 263, n. 1), from which it differs in the shape of the leaves, in their mode of insertion, and, especially, in the peculiar characters of the amphigastria.

**P. lævigata** Lindb. (*Madotheca lævigata* Dum. Syn. Hep. p. 276). East Maui (Baldwin, 222!).

**Pleurozia gigantea** Lindb. (*Physiotium sphagnoides* Nees, Syn. Hep. p. 235). Hawaii (Herb. Hook.).

Var. major (Jack), Hedwigia, xxv, p. 65. Hawaii (Herb. Carrington).

**P. cochleariformis** Dum. (*Physiotium cochleariforme* Nees, Syn. Hep. p. 235). Hawaii (Herb. Hook.).

**P. conchæfolia** Aust. (*Physiotium conchæfolium* Syn. Hep. p. 235). Hawaiian Islands (Beechey, etc.); West Maui (Baldwin, 18!).

**P. subinflata** Aust. (*Physiotium subinflatum* Aust. Proc. Acad. Nat. Sci. Phil., Dec. 1869). Hawaii (Mann and Brigham); West Maui (Baldwin, 47!).

**Trichocolea gracillima** Aust. Bot. Gazette, iii, p. 6, West Maui (Baldwin, 134!).

**Herberta sanguinea** Aust. Bull. Torr. Bot. Club, vi, p. 302 (*Sendtnera juniperina*, var. *sanguinea* Mont. Syn. Hep. p. 239). East and West Maui (Baldwin, 15! 92! 98!).

**Mastigophora gracilis** Mont. (*Sendtnera gracilis*, Syn. Hep. p. 243; *S. tristicha* Aust. Proc. Acad. Nat. Sci. Phil., Dec. 1869). Oahu (Mann and Brigham! Baldwin, 274!).

**Bazzania? integrifolia** (*Mastigobryum? integrifolium* Aust. Bot. Gazette, i, p. 32). Hawaiian Islands (Baldwin).

**B. patens** (*M. patens* Lindenb. Syn. Hep. p. 221; *M. parvistipulum* Aust. Bull. Torr. Bot. Club, v, p. 16). Oahu (Mann and Brigham); West Maui (Baldwin, 68!).

**B. Brighami** (*M. Brighami* Aust. l. c.). Oahu (Mann and Brigham); Hawaiian Islands (Baldwin!).

**B. cordistipula** (*M. cordistipulum* Lindenb. Syn. Hep. p. 224). Hawaiian Islands (Gaudichaud, etc.): Oahu (Mann and Brigham); Molokai (Baldwin, 222!)

**B. falcata** (*M. falcatum* Lindenb. Syn. Hep. p. 231). West Maui (Baldwin, 12!).

**B. Baldwinii** Aust. Ms. West Maui (Baldwin, 199!).

Stems simple or slightly branched, flagelliferous, clustered; leaves approximate or slightly imbricated, usually somewhat deflexed, triangular-ovate, entire, the apex rounded or variously 1–4-toothed, the dorsal margin arching over the stem, the ventral oblique or somewhat cordate at its insertion; amphigastria a little wider than the stem, orbicular-quadrate, entire or crenulate above.

Stems 3–4$^{cm}$ long; leaves 0·7–0·8$^{mm}$ long by 0·4–0·6$^{mm}$ wide; leaf-cells averaging about 0·024$^{mm}$ in diameter.

**B. deflexa** Underw. (*M. deflexum* Nees, Syn. Hep. p. 231). East Maui (Baldwin, 231!).

**B. minuta** (*M. minutum* Aust. Bull. Torr. Bot. Club, v, p. 17). Hawaiian Islands (Hillebrand); East Maui (Baldwin, 65 in part!).

**Lepidozia Sandvicensis** Lindenb. Syn. Hep. p. 201 (*L. filipendula* Tayl. Syn. Hep. p. 713). Hawaiian Islands (Tolmie, etc.); West Maui (Baldwin, 69!).

**L. reptans** Nees. West Maui (Baldwin, 150!).

**Cephalozia multiflora** Spruce. Oahu (Mann and Brigham).

**C. Sandvicensis** (*Jungermannia Sandvicensis* Mont. Syn. Hep. p. 142). Hawaiian Islands (Gaudichaud).

**Odontoschisma subjulacea** Aust. Bull. Torr. Bot. Club, vi, p. 303 (*J. caudifera* Tayl. Mss., in part). West Maui (Baldwin, 233!).

**O. Sandvicensis** (*Sphagnœcetis Sandvicensis* Ångstr. Öfver. af Kongl. Vet.-Akad. Förhandl., 1872, No. 4, p. 22). Hawaiian Islands (Andersson).

**Kantia bifurca** (*Calypogeia bifurca* Aust. Proc. Acad. Nat. Sci. Phil., Dec. 1869). Hawaiian Islands (Hillebrand).

**K. bidentula** (*C. bidentula* Nees, Syn. Hep. p. 199). West Maui (Baldwin, 101!).

**K. Baldwinii** (*C. Baldwinii* Aust. Bot. Gazette, i, p. 32). West Maui (Baldwin, 141!).

**Saccogyna? Bolanderi** (*Gymnanthe? Bolanderi* Aust., Proc. Acad. Nat. Sci. Phil., Dec. 1869). Hawaiian Islands (Hillebrand).

**Scapania undulata** Dum. var. West Maui (Baldwin, 94 in part!).

**S. Oakesii** Aust.? West Maui, young specimens (Baldwin, 94 in part!).

**S. nemorosa** Dum. East and West Maui, a small form (Baldwin, 149, 214!).

**S. planifolia** Dum. West Maui (Baldwin, 83!).

**Diplophyllum albicans** Dum. (*Jungermannia albicans* Linn. Syn. Hep. p. 75). East Maui (Baldwin, 245!).

**Lophocolea connata**, var. *β?* Syn. Hep. p. 153. West Maui (Baldwin, 66!). The specimens are all sterile and may possibly be *L. Martiana* Nees.

**L. Breutelii** Gottsche, Syn. Hep. p. 154. West Maui, mixed with the preceding species!

**L. Columbica** Gottsche, Syn. Hep. p. 155. West Maui (Baldwin, 91, in part).

**L. Orbigniana** Mont. et Nees, Syn. Hep. p. 156. West Maui (Baldwin, 76!).

**L. Gaudichaudii** Mont. Syn. Hep. p. 156. Hawaiian Islands (Gaudichaud); West Maui (Baldwin, 111!).

**L. bidentata** Dum. Syn. Hep. p. 159. West Maui (Baldwin, 140!).

**L. Beecheyana** Tayl. Syn. Hep. p. 690. Oahu (Beechey in Herb. Hook.).

**L. spinosa** Gottsche, Syn. Hep. p. 170. Hawaii (Herb. Hook.).

**Plagiochila simplex** Lindenb. Syn. Hep. p. 30. East and West Maui (Baldwin, 117! 184! 196! 205! 237!).

**P. gracillima** Aust. Ms. Molokai (Baldwin, 211!).

Much branched, glossy, and bearing numerous flagellæ; leaves of stem and main branches distant, oblong-ovate, the dorsal margin curved, usually entire, slightly decurrent at base, the ventral either entire or bearing 1 or 2 sharp teeth, the apex strongly emarginate-

bidentate with sub-equal divergent teeth; leaves of flagellæ minute, mostly bidentate.

Leaves $0.8^{mm}$ long, $0{\cdot}45^{mm}$ wide; cells of leaves averaging $0{\cdot}018^{mm}$ in diameter.

**P. frondescens** Nees, Syn. Hep. p. 31. East Maui (Baldwin, 5, 85!).

**P. Gaudichaudii** Mont. et Gottsche, Annal. des Sc. Nat. 4me sèrie, T. vi, p. 193 (*P. tenuis* Mont. non Lindenb.). Hawaiian Islands (Gaudichaud, Andersson).

**P. Baldwinii** Aust. Ms. (*P. flava* Aust. Ms.). West Maui (Baldwin, 115!).

Loosely cæspitose, brownish-yellow, robust; stems simple or sparingly branched; leaves sub-imbricated, ovate, slightly curved, the margins entire or a little undulate, the dorsal decurrent; the apex rounded, usually bearing from one to three sharp teeth.

Stem $5–8^{cm}$ long; leaves $2–2{\cdot}5^{mm}$ long, $1^{mm}$ wide; cells $0{\cdot}027^{mm}$ in diameter.

**P. fissidentoides** Tayl. Syn. Hep. p. 636. Hawaiian Islands (Menzies); West Maui (Baldwin, 95).

**P. adiantoides** Lindenb. Syn. Hep. p. 38. Oahu (Mann and Brigham).

**P. deflexa** Mont et Gottsche, Annal. des. Sc. Nat. 4me sèrie, T. vi, p. 192 (*P. patula* Mont. non Nees et Mont.; *P. patens* Aust. Ms.). Hawaiian Islands (Gaudichaud); West Maui (Baldwin, 60!).

**P. Owaihiensis** Nees et Lindenb. Syn. Hep. p. 46. Hawaii (Herb. Hook.); Oahu, West Maui, Kauai (Baldwin, 160! 201! 206!).

**P. Eatoni** Aust. Ms. West Maui (Baldwin, 109!).

Stems mostly simple; leaves opposite, loosely imbricated, spreading, slightly unsymmetrical (the leaves on one side being more obliquely inserted than on the other), orbicular-ovate, entire, the dorsal margins decurrent, the ventral connate at insertion.

Stems $10–12^{cm}$ long; leaves $1{\cdot}5–2^{mm}$ long, $1–1{\cdot}5^{mm}$ wide. *P. Eatoni* is apparently a near ally of *P. Brauniana* Nees (Syn. Hep. p. 51, n. 70) but the leaves are distinctly imbricated and somewhat longer than they are broad.

**P. oppositifolia** Aust. Bull. Torr. Bot. Club, v, p. 16. Hawaiian Islands (Hillebrand); West Maui (Baldwin, 93! 97!).

**P. biserialis** Lehm. et Lindenb. Syn. Hep. p. 53. East Maui (Baldwin, 216!).

**P. deltoidea** Lindenb. Syn. Hep. p. 55. West Maui (Baldwin, 198!).

**P. acutiuscula** Aust. Ms. West Maui (Baldwin, 116!).

Pale brownish-green, cæspitose, glossy; stems simple; leaves imbricated, spreading, ovate, the margins entire except at the apex,

which is sharply incised-dentate, the apical tooth being larger than the others and projecting beyond them.

Stems 2·5–4$^{cm}$ long; leaves 1$^{mm}$ wide, 1·5–2$^{mm}$ long; leaf-cells averaging 0·033$^{mm}$ in diameter.

Several other possibly distinct *Plagiochilæ* occur in too small quantity for determination.

**Mylia Taylori** S. F. Gray (*Jungermannia Taylori* Hook. Syn Hep. p. 82). Hawaii (Herb. Lehm.).

**Jungermannia piligera** Nees, Syn. Hep. p. 81. Oahu (Meyen); West Maui (Baldwin, 79!).

**J. rigida** Aust. Proc. Acad. Nat. Sci. Phil., Dec. 1869. Hawaiian Islands (Hillebrand); West Maui (Baldwin, 64! 154!).

**J. robusta** Aust. l. c. Hawaiian Islands (Hillebrand); West Maui (Baldwin, 268!).

**J. coriacea** Aust. l. c. Hawaiian Islands (Hillebrand, Baldwin!).

**J. macrophylla** Ångstr. Öfver. af. Kongl. Vet.-Akad. Förhandl. 1872, No. 4, p. 22. Hawaiian Islands (Andersson).

**J. subulata** n. sp. Hawaiian Islands (Baldwin!).

Cæspitose, brownish-green; stems mostly simple, creeping, ascending at the apex, radiculose; leaves spreading, imbricated, ovate, entire, rounded at the apex; amphigastria wanting; outer involucral leaves like the stem-leaves, the inner very small, awl-shaped, slightly denticulate; perianth (young), oblong-obovate, contracted at the denticulate mouth, surpassing the inner involucral leaves, scarcely, if at all, plicate.

Leaves 2–2·5$^{mm}$ long, 1–1·5$^{mm}$ wide; cells oval, about 0·029$^{mm}$ in width, those of the inner involucral leaves rapidly increasing in size at the apex and resembling in character the terminal cells of the perianth.

**J. lurida** Dum. (*J. nana* Nees, Syn. Hep. p 91). Hawaiian Islands (Hillebrand).

**J. Esenbeckii** Mont. Syn. Hep. p. 98. Hawaiian Islands (Gaudichaud, Andersson).

**J. lucens** n. sp. East Maui (Baldwin, 65, in part!).

Loosely tufted, whitish-green; stems simple or innovating from beneath the involucre, rootless; leaves remote, except at the summit of the stem where they are imbricated, loosely areolate, pellucid, sub-transverse, entire, complicate, bilobed about one-third their length, the lobes acute and nearly equal with an acute sinus; involucral leaves about three, deeply two-lobed, the margins irregularly

sinuous-denticulate; perianth ovate-cylindrical, somewhat plicate at the dentate mouth.

Stems 1·5–2·5$^{cm}$ long; leaves 0·5–0·75$^{mm}$ long, 0·4–0·7$^{mm}$ wide; leaf-cells averaging 0·055$^{mm}$ long by 0·035$^{mm}$ wide.

This species is allied to *J. minuta* Crantz; it differs in its larger size, in the larger and looser areolation of its leaves, in its pale color, and in its normally two-cleft involucral leaves. The sinus of the leaves has an angle of a little less than 90°; the sinus of the involucral leaves is often much sharper.

**Nardia callithrix** Spruce (*J. callithrix* Lindenb. et Gottsche, Syn. Hep. p. 673). Hawaiian Islands (Hillebrand).

**N. Mauii** (*J. Mauii* Aust. Bull. Torr. Bot. Club, vi, p. 303). West Maui (Baldwin, 242!).

**N. exserta** n. sp. West Maui (Baldwin, 264!).

Diœcious, cæspitose, dark or blackish green; stems simple, radiculose, rigid, erect; leaves subtransversely inserted, imbricated, obliquely spreading, orbiculate, entire; amphigastria none; involucral leaves two or three, similar to the stem-leaves, slightly connate with the perianth at its base; perianth large, long-exserted, obovoid or campanulate, slightly plicate, the mouth open, lacerate.

Stems 2–3$^{cm}$ long; leaves about 1$^{mm}$ in diameter, the cells averaging 0·025$^{mm}$ in diameter; perianth about 3$^{mm}$ in length.

**Tylimanthus integrifolius** n. sp. East and West Maui (Baldwin, 95! 183!).

Diœcious, cæspitose, brownish-green; stems simple or somewhat pinnately divided, eradiculose except near the extremity of fruiting stems; leaves contiguous or slightly imbricated, oblong or ovate, the margins mostly entire, except near the involucre where they are irregularly crenulate, the dorsal margin often more or less indented near the rounded apex; amphigastria none; involucre ovoid, thickened, with numerous radicles.

Stems about 4$^{cm}$ long; leaves 1–1·5$^{mm}$ long, 1$^{mm}$ wide; cells 0·025$^{mm}$ in diam. in middle of leaf, longer towards base; involucre 2·5–3$^{mm}$ long, 1$^{mm}$ wide.

**Pallavicinia cylindrica** (*Steetzia cylindrica* Aust. Bull. Torr. Bot. Club, v, p. 17). Hawaiian Islands (Hillebrand); West Maui (Baldwin, 70 in part!).

**P. Baldwinii** (*S. Baldwinii* Aust. l. c. vi, p. 303). West Maui (Baldwin, 70 in part).

**Symphyogyna semi-involucrata** Aust. l. c. v, p. 15. Oahu (Mann and Brigham); Hawaiian Islands (Baldwin!).

**Metzgeria dichotoma** Nees, Syn. Hep. p. 504. West Maui (Baldwin, 106!).

**Aneura multifida** Dum. Syn. Hep. p. 496. Oahu (Mann and Brigham); Hawaiian Islands (Hillebrand).

**A. pinnatifida** Nees, Syn. Hep. p. 495. West Maui (Baldwin, 110!).

**A. palmata** Dum. Syn. Hep. p. 498. Oahu (Mann and Brigham); Hawaiian Islands (Hillebrand).

**A. pectinata** Aust. Bull. Torr. Bot. Club, v, p. 15. Oahu (Mann and Brigham); Hawaiian Islands (Hillebrand); West Maui (Baldwin, 71!).

**A. pinguis** Dum. Syn. Hep. p. 493. West Maui (Baldwin, 119!).

**Dendroceros Clintoni** Aust. Bull. Torr. Bot. Club, v, p. 14. Hawaiian Islands (Mann and Brigham).

**Anthoceros Vincentianus** Lehm. et Lindenb. Syn. Hep. p. 587. Kauai (Baldwin, 238!).

**A. vesiculosus** Aust. Bull. Torr. Bot. Club, v, p. 17. Hawaiian Islands (Hillebrand, Baldwin!).

**Marchantia polymorpha** Linn. Syn. Hep. p. 522. Hawaiian Islands (Hillebrand).

**M. crenata** Aust. Bull. Torr. Bot. Club, v, p. 14. Hilo (Mann and Brigham!).

**M. disjuncta** Sull. Mem. Amer. Acad. n. ser. iii, p. 63. Hawaiian Islands (Baldwin, 90! 145 in part!).

The male receptacles agree with Sullivant's description and figures, but the female are not so deeply parted as he represents.

Several other species of *Marchantia*, which were considered new and named provisionally by Austin, occur in too small quantity for description.

**Fimbriaria innovans** Aust. (*Marchantia innovans* Aust. Bull. Torr. Bot. Club, v, p. 14). Hanalei, Kauai (Mann and Brigham); East Maui (Baldwin, 89!).

**Dumortiera hirsuta** Nees, Syn. Hep. p. 543. Hawaiian Islands (Hillebrand); Maui (Mann and Brigham, Baldwin!).

**D. trichocephala** Nees, Syn. Hep. p. 545. Hawaiian Islands (Douglas); Oahu (Mann and Brigham).

**D. Nepalensis** Nees. Hawaiian Islands (Hillebrand).

**Aitonia cordata** (*Plagiochasma cordata* Lehm. et Lindenb. Syn. Hep. p. 512). Hawaiian Islands (Hillebrand).

## DESCRIPTION OF FIGURES.

### PLATE XXII.

*Porella Hawaiiensis*, n. sp. Fig. 1. Plant × ½; fig. 2, branch, ventral view × 12; fig. 3, pair of leaves, dorsal view × 12.

*Bazania Baldwinii* Aust. Ms. Figs. 4, 5. Parts of stems, dorsal and ventral views × 12.

*Plagiochila gracillima* Aust. Ms. Figs. 6, 7. Parts of stems, dorsal and ventral views × 12.

*P. Baldwinii* Aust. Ms. Fig. 8. Stem × ½: figs. 9, 10, parts of stems, dorsal and ventral views × 6.

*P. Eatoni* Aust. Ms. Figs. 11, 12. Parts of stems, dorsal and ventral views × 6.

*P. oppositifolia* Aust Fig. 13. Plant × ½; figs. 14, 15, parts of stems, dorsal and ventral views × 6; fig. 16, involucral leaf × 6; fig. 17, perianth × 6.

### PLATE XXIII.

*Plagiochila acutiuscula* Aust. Ms. Figs. 1, 2. Parts of stems, dorsal and ventral views × 6.

*Jungermannia subulata*, n. sp. Fig. 3. Extremity of fruiting stem with perianth × 6; fig. 4, involucral leaf × 75.

*J. lucens*, n. sp. Fig. 5. Fertile stem × ½; fig. 6, extremity of same × 6; fig. 7, extremity of sterile stem × 6; fig. 8, leaf expanded × 6; fig. 9, involucral leaves × 6; fig. 10, perianth × 6.

*Nardia exserta*, n. sp. Fig. 11. Extremity of fertile stem, showing perianth × 6.

*Tylimanthus integrifolius*, n. sp. Fig. 12. Extremity of fertile plant, showing involucre × 6; figs. 13, 14, parts of sterile stems, dorsal and ventral views × 6.

## XVI.—An Arrangement of the Genera of Hepaticæ. By A. W. Evans.

At the beginning of the present century, the writers on Hepaticæ included nearly the whole of the present order Jungermanniaceæ under the single genus *Jungermannia*. The only exception to this rule was in the case of *Blasia pusilla*, which was usually kept as a distinct genus; but even this was reduced to a species of *Jungermannia* by Hooker in his "British Jungermannieæ" finished in 1816. The splitting-up of this vast genus *Jungermannia* was begun by Raddi (1820), who, in an account of some of the Italian Hepaticæ, arranged his species under a dozen or more distinct genera. He was quickly followed in this work of division by S. F. Gray (1821) and by Dumortier (1822). Each of these three investigators worked independently, and, in this way, gave rise to a mass of synonymy which has since caused a great deal of confusion. The work of Gray, which remained overlooked by botanists for more than forty years, has been especially unfortunate in this respect; and this chiefly on account of the generic names which he employed. In nearly every case, these were derived from the names of persons, but, instead of using the feminine termination, as is usual in such cases, Gray wrote his names in the masculine, and published such genera as Bazzanius, Kantius, etc. Most recent writers have, nevertheless, adopted these names in an emended form, the termination being changed into the feminine.

In the Synopsis Hepaticarum (1844–47), most of the genera of Raddi and Dumortier were acknowledged; but, in some cases, the names adopted for these genera were the later ones of Corda (1829) and of Nees von Esenbeck (1833–38), so that they ought no longer to be retained. Since the publication of this volume, there has been no very comprehensive work on descriptive hepaticology. The numerous shorter works on the species of limited regions or of special groups, which have appeared from time to time, have, however, contained a number of new genera; and, since many of these papers appeared in local periodicals or in the transactions of scientific societies, they are now difficult of access.

Taking these facts into consideration, it has been thought well to bring together, in an arrangement, the various genera which are

acknowledged by most recent hepaticologists. Under each genus, the place and the date of its original publication have been given, together with enough of the synonymy to show the claims of the accepted name for recognition. The names of Gray are in all cases given with the feminine termination.

The arrangement adopted is based on that of Dr. Underwood in the Sixth Edition of Gray's Manual. It combines peculiarities of both Lindberg's and Spruce's arrangements. The tribal names are mostly taken from Lindberg.

In the preparation of this and the preceding paper, my sincere thanks are due Prof. D. C. Eaton, who has allowed me the use of his library and herbarium, and who has given me much bibliographical and critical assistance.

## ORDER I. JUNGERMANNIACEÆ.

### TRIBE I. FRULLANIEÆ.

1. **Frullania** Raddi, Jung. Etr. in Mem. Moden. xviii, p. 30 (1820): Dum Rev. des Genres, p. 12; Hep. Eur. p. 26: G. L. & N. Syn. Hep. p. 408: Spruce, Hep. Amaz. et And. in Trans. Bot. Soc. Edin. xv, p. 3.
**Jubula** Dum. Comm. bot. p. 112 (1822), in part.
**Jubula**, sect **Ascolobia** Dum. Syll. Jung. p. 36.

Widely distributed; species about 150. The genus is divided by Spruce (Hep. Amaz. et And.) into six subgenera:—*Chonanthelia*, *Trachycolea*, *Homotropantha*, *Meteoriopsis*, *Thyopsiella* and *Diastaloba*.

2. **Jubula** Dum. Comm. bot. p. 112 (1822), in part.
**Jubula**, sect. **Jubulotypus** Dum. Syll. Jung. p. 36.
**Jubula** Dum. Rev. des Genres, p. 12 (1835); Hep. Eur. p. 26: Spruce, Hep. Amaz. et And. p. 59. Underwood in Gray's Manual, Ed. vi, p. 708.
**Frullaniæ** sp. G. L. & N. Syn. Hep. p. 426.

Consists of 2 species,—the variable *J. Hutchinsiæ* Dum. of Europe and America and *J. piligera* (Aust.) of the Pacific, which is perhaps a variety of the first.

3. **Lejeunea** Lib. in Ann gen. sc. phys. T. 5, p. 372 (1820): Dum. Comm. bot. p. 111; Syll. Jung. p. 32; Rev. des Genres, p. 11; Hep. Eur. p. 18: G. L. & N. Syn. Hep. p. 308.

As now defined by most hepaticologists, the genus includes—

**Marchesinia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 689 (1821), (**Phragmicoma** Dum. Comm. bot p. 112; Syll. Jung. p. 35; Rev. des Genres, p. 13; Hep. Eur. p. 30: G. L. & N. Syn. Hep. p. 292);
**Colura** Dum. Rev. des. Genres, p. 12;
**Omphalanthus** Lindenb. & Nees in Syn. Hep. p. 303;

**Ptychanthus** Nees, Hep. Eur. iii, p. 105: G. L. & N. Syn. Hep. p. 289;
**Thysananthus** Lindenb. in Syn. Hep. p. 286;
**Bryopteris** Lindenb. l. c. p. 284.

The genus *Lejeunea* is very widely distributed but is especially abundant in tropical regions. As first published by Mademoiselle Libert, it contained 2 species,—*L. calcarea* and *L. serpyllifolia* of Europe and America; in its present extended sense it is the largest genus of Hepaticæ, and contains 300 to 400 species. The genus is divided by Spruce (Hep. Amaz. et And.) into 2 sections and 38 subgenera, as follows:—§ 1. **Holostipæ**, comprising the subgenera *Stictolejeunea*, *Neurolejeunea*, *Peltolejeunea*, *Omphalolejeunea*, *Archilejeunea*, *Ptycholejunea*, *Mastigolejeunea*, *Thysanolejeunea*, *Dendrolejeunea*, *Bryolejeunea*, *Acrolejeunea*, *Lopholejeunea*, *Platylejeunea*, *Anoplolejeunea*, *Brachiolejeunea*, *Homalolejeunea*, *Dicranolejeunea* and *Odontolejeunea*. § 2. **Schizostipæ**, comprising the subgenera *Prionolejeunea*, *Crossotolejeunea*, *Harpalejeunea*, *Trachylejeunea*, *Drepanolejeunea*, *Leptolejeunea*, *Ceratolejeunea*, *Taxilejeunea*, *Macrolejeunea*, *Otigoniolejeunea*, *Hygrolejeunea*, *Euosmolejeunea*, *Pycnolejeunea*, *Potamolejeunea*, *Cheilolejeunea*, *Eulejeunea*, *Microlejeunea*, *Cololejeunea*, *Diplasiolejeunea* and *Colurolejeunea*.

4. **Myriocolea** Spruce, Hep. Amaz. et And. p. 305 (1884).

Contains a single species, *M. irrorata* Spruce of South America.

5. **Radula** Dum. Comm. bot. p. 112 (1822), in part.
**Radula**, sect. **Radulotypus** Dum. Syll. Jung. p. 38 (1831).
**Radula** Dum. Rev. des Genres, p. 14 (1835); Hep. Eur. p. 31 (1874): G. L. & N. Syn. Hep. p. 253.
**Candollea** Raddi, Jung. Etr. in Mem. Moden. xviii, p. 24 (1820), in part, not **Candollea** Labil.
**Martinellia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 690 (1821), in part.

A widely diffused genus of about 75 species, the typical one being the common *R. complanata* Dum. The genera *Radula* and *Martinellia*, as first proposed by their authors, included the genera *Radula*, *Scapania* and *Plagiochila*, as these are now understood. In his Sylloge (pp. 37–43), Dumortier divided his original *Radula* into three sections:—*Radulotypus*, *Scapania* and *Plagiochila*; these he soon elevated into the genera *Radula*, *Scapania* and *Plagiochila* with almost their present limitations (Rev. des Genres, p. 14). If we supersede any of Dumortier's names by the older *Martinellia* of Gray, it should be *Radula*, since the first of Gray's species of *Martinellia* belong to that genus. As, however, Gray's genus is so loosely defined, and Dumortier's names are in so universal use, it is

best to retain the latter, in accordance with the views of most authors. (See Carrington, Brit. Hep. p. 52.)

6. **Porella** Dill. Hist. Musc. p. 459 (1741): Lindb. in Act. Soc. Sc. Fenn. ix, pp. 329–345 (1869).

**Madotheca** Dum. Comm. bot. p. 111 (1822); Syll. Jung. p. 30; Rev. des Genres, p. 11: G. L. & N. Syn. Hep. p. 262.

**Cavendishia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 689, not **Cavendishia** Lindl.

**Bellincinia** and **Antoiria** Raddi, Jung. Etr. in Mem. Moden. xviii, pp. 18, 19.

A widely distributed genus of 75–100 species. The original species is *P. porella* of Linnæus, which both he and Dillenius placed among the Musci; here it remained overlooked, until it was brought to light by Lindberg.

7. **Pleurozia** Dum. Rev. des Genres, p. 15 (1835); Hep. Eur. p. 52.

**Radulæ** sp. Dum. Syll. Jung. p. 38.

**Physiotium** Nees, Eur. Leberm. iii, p. 75 (1838): G. L. & N. Syn. Hep. p. 234: Jack, Hedwigia, xxv, p. 49.

Jack enumerates 10 species in his monograph of the genus; of these one only, *P. cochleariformis*, occurs in Europe, the others being confined to Asia and the Pacific Islands.

## TRIBE II. PTILIDIEÆ.

8. **Ptilidium** Nees, Eur. Leberm. i, p. 95 (1833): G. L. & N. Syn. Hep. p. 249.

**Jungermannia**, sect. **Blepharozia** Dum. Syll. Jung. p. 46 (1831).

**Blepharozia** Dum. Rev. des Genres, p. 16 (1835).

A genus of about 8 species, the typical *P. ciliare* Nees being widely distributed.

9. **Trichocolea** Dum. Comm. bot. p. 113 (1822); Syll. Jung. p. 66; Rev. des Genres, p. 20; Hep. Eur. p. 111: G. L. & N. Syn. Hep. p. 236.

A small but widely distributed genus of which the type is *T. tomentella* Dum.

The name of the genus was first published by Dumortier in his Commentationes as "*Thricolea*," which spelling he retained in his Sylloge. Nees von Esenbeck corrected the spelling to "*Trichocolea*" (Eur. Leberm. iii, p. 105); but Dumortier, objecting to the sound of this name, shortened it into "*Tricholea*" both in his Revision des Genres and in his Hepaticæ Europæ. As, however, the name "*Trichocolea*" is preserved by most authors, it has been retained here.

10. **Leiomitra** Lindb. in Act. Soc. Sc. Fenn. x, 515 (1875).

Lindberg gives 2 species,—*L. tomentosa* of tropical America and *L. capillata* of the Philippine Islands; several additional species are described by Spruce.

11. **Chætocolea** Spruce, Hep. Amaz. et And. p. 346 (1885).

Contains a single South American species.

12. **Lepidolæna** Dum. Rev. des Genres, p. 13 (1835): Spruce, Hep. Amaz. et And. p. 336.

**Polyotus** Gottsche in G. L. & N. Syn. Hep. p. 13 (1845).

A beautiful genus of the Southern Hemisphere; species 10–15, among which may be mentioned *L. Menziesii*, *L. clavigera* and *L. palpebrifolia*, the original species.

13. **Herberta** S. F. Gray, Nat. Arr. Br. Pl. i, p. 705 (1821): Spruce, Hep. Amaz. et And. p. 340. Underw. in Gray's Manual, Ed. vi, p. 709.

**Schisma** Dum. Comm. bot. p. 114 (1822); Syll. Jung. p. 76; Rev. des. Genres p. 23; Hep. Eur. p. 123.

**Sendtnera** Nees in G. L. & N. Syn. Hep. p. 238 (1845).

Widely distributed; species 10–15; the genus is represented in both Europe and America by *H. adunca* Gray.

14. **Lepicolea** Dum. Rev. des Genres, p. 20 (1835): Lindb. in Act. Soc. Sc. Fenn. x, p. 516.

**Leperoma** Mitt in Hook f. Handb. N. Z. Fl. pp. 751, 754 (1867).

Mostly in the Southern Hemisphere; species 3,—*L. scolopendra* Dum., *L. ochroleuca* Lindb. and *L. pruinosa* Spruce.

15. **Mastigophora** Nees, Eur. Leberm. iii, p. 95 (1833): Mitt. in Hook. f. Handb. N. Z. Fl. p. 754.

**Sendtnera**, sect. **Mastigophora** G. L. & N. Syn. Hep. 241.

Species 10–15, scattered; *M. Woodsii* is the only European species.

16. **Isotachis** Mitt. in Hook. f. Handb. N. Z. Fl. p. 526 (1867): Spruce, Hep. Amaz. et And. p. 337.

Species 10–15, mostly in the Southern Hemisphere.

## TRIBE III. LEPIDOZIEÆ.

17. **Lembidium** Mitt. in Hook. f. Handb. N. Z. Fl. p. 754 (1867).

A small genus confined to the islands of the South Seas; 3 species are known,—*L. nutans* Mitt. of New Zealand, *L. ventrosum* Mitt. of Kerguelen Land, and *L. dendroides* Carrington & Pearson of New South Wales.

18. **Mytilopsis** Spruce, "On Cephalozia" (1882); Hep. Amaz. et And. p 387.

Contains one South American species.

19. **Micropterygium** Lindenb. in G. L. & N. Syn. Hep. p. 233 (1845).

Species 5–10, mostly in tropical America.

20. **Bazzania** S. F. Gray, Nat. Arr. Br. Pl. i, p. 704 (1821): Spruce, Hep. Amaz. et And. p. 366: Underw. Cat. Hep. p. 82.
**Pleuroschisma,** sect. **Pleuroschismotypus** Dum. Syll. Jung. p. 70 (1831).
**Pleuroschisma** Dum. Rev. des Genres, p. 19 (1835); Hep. Eur. 102.
**Herpetium,** sect. **Mastigobryum** Nees, Hep. Eur. iii, p. 43.
**Mastigobryum** Nees in G. L. & N. Syn. Hep. p. 214.

Species 100–125, especially numerous in tropical regions; the commonest northern species is *B. trilobata* S. F. Gray.

21. **Sprucella** Steph. in Engler's Bot. Jahrb. viii, p. 92 (1887); Hedwigia, xxx, p. 215.

Species 1, *S. succida* (*Lepidozia succida* Mitt.) of western Africa.

22. **Lepidozia** Dum. Rev. des Genres, p. 19 (1835); Hep. Eur. p. 109: G. L. & N. Syn. Hep. p. 200.
**Pleuroschisma,** sect. **Lepidozia** Dum. Syll. Jung. p. 69.
**Herpetium,** sect. **Lepidozia** Nees, Hep. Eur. iii, p. 31.

Species 50–75, most abundant in tropical regions; the typical *L. reptans* Dum. is the most frequent northern species.

23. **Arachniopsis** Spruce, "On Cephalozia" (1882); Hep. Amaz. et And. p. 354.

Contains 3 South American species.

24. **Cephalozia** Dum. Rev. des Genres, p. 19 (1835); Hep. Eur. p. 87: Spruce, "On Cephalozia"; Hep. Amaz. et And. p. 388.
**Jungermannia,** sect. **Cephalozia** Dum. Syll. Jung. p. 60.
**Jungermannieæ** sp. G. L. & N. Syn. Hep. p. 131.
**Zoöpsis** Hook. f. & Tayl. Crypt. Ant. p. 55 (1845).
**Trigonanthus** Spruce, Trans. Bot. Soc. Edin. iii, p. 207 (1850).

A widely distributed genus of 40–50 species; it may be divided into the following subgenera (most which were first proposed by Dr. Spruce):—*Protocephalozia, Pteropsiella, Zoöpsis, Alobiella, Eucephalozia, Cephaloziella* and *Prionolobus.*

25. **Herpocladium** Mitt. in Jour. Linn. Soc. xv, p. 69 (1877).

A small genus of the South Pacific.

26. **Odontoschisma** Dum. Rev. des Genres, p. 19 (1835).
**Pleuroschisma,** sect. **Odontoschisma** Dum. Syll. Jung. p. 68.
**Sphagnoecetis** Nees in G. L. & N. Syn. Hep. p. 148 (1845).
**Cephalozia,** sect. **Odontoschisma** Spruce, "On Cephalozia."

Species 10–15, scattered; the typical *O. Sphagni* occurs in both Europe and America.

27. **Hygrobiella** Spruce, "On Cephalozia" (1882).

Species 3,—*H. laxifolia, H. myriocarpa* and *H. Nevicensis,* all of northern regions.

28. **Pigafettoa** Mass. in Nuovo Gior. Bot. Ital. xvii, p. 237 (1885).

Consists of a single Patagonian species, *P. crenulata.*

29. **Pleuroclada** Spruce, "On Cephalozia."

Species 2,—*P. albescens* and *P. Islandica* of Arctic regions.

30. **Anthelia** Dum. Rev. des Genres, p. 18 (1835); Hep. Eur. p. 97: Spruce, "On Cephalozia."

**Jungermannia,** sect. **Anthelia** Dum. Syll. Jung. p. 63.

Contains 4 species, mostly of northern regions; *A. julacea* Dum. is the typical species.

31. **Blepharostoma** Dum. Rev. des. Genres, p. 18 (1835); Hep. Eur. p. 94: Spruce, "On Cephalozia": Underw. Cat. Hep. p. 80.

**Jungermannia,** sect. **Blepharostoma** Dum. Syll. Jung. p. 18.

**Jungermanniæ** sp. G. L. & N. Syn. Hep. p. 144.

**Chætopsis** Mitt. Jour. Linn. Soc. viii, p. 51 (1864).

Contains 2 species,—the widely distributed *B. trichophyllum* Dum. and *B. palmatum* Lindb. of Australia and New Zealand.

32. **Chandonanthus** Mitt. in Hook. f. Handb. N. Z. Fl. p. 753 (1867): Lindb. in Act. Soc. Sc. Fenn. x, p. 517 (1875).

**Anthelia** Dum. in part.

Includes *C. setiformis* Lindb. of northern regions, *C. squarrosa* of New Zealand, and a few other species.

33. **Adelanthus** Mitt. in Jour. Linn. Soc. vii, p. 243 (1863): Dum Hep. Eur. p. 46.

The original species of Mitten were *A. falcatus*, *A. Magellanicus*, *A. Lindbergianus* and *A. decipiens.* Since his publication of the genus a few other species have been added. The genus is mostly tropical, but *A. decipiens* occurs in Ireland.

34. **Anomoclada** Spruce, Jour. of Bot. xiv (1876); Hep. Amaz. et And. p. 407.

Species 1, *A. mucosa* of South America.

TRIBE IV. SACCOGYNEÆ.

35. **Kantia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 706 (1821): Underw. in Gray's Manual, Ed. vi, p. 713.

**Cincinnulus** Dum. Comm. bot. p. 113 (1822); Syll. Jung. p. 72; Rev. des Genres, p. 21; Hep. Eur. p. 115.

**Calypogeia** (sect. B) Raddi, Jung. Etr. in Mem. Mod. xviii, p. 44: G. L. & N. Syn. Hep. p. 198.

Species 10–15, scattered; the typical *K. Trichomanis* occurring in both Europe and America.

36. **Saccogyna** Dum. Comm. bot. p. 113 (1822); Syll. Jung. p. 74; Rev. des Genres, p. 21; Hep. Eur. 117: G. L. & N. Syn. Hep. p. 194.
**Sykorea** Corda in Opiz. Naturl. p. 652 (1829).

Species 3,—*S. viticulosa* Dum. of Europe, *S. australis* Mitt. of the South Pacific, and *S. jugata* Mitt. of Samoa.

37. **Geocalyx** Nees, Eur. Leberm. i, p. 97 (1833): Dum. Rev. des Genres, p. 22; Hep. Eur. p. 118: G. L. & N. Syn. Hep. p. 194.

Contains the common *G. graveolens* Nees and a doubtful West Indian species. The genus is included by Carrington and Lindberg under *Saccogyna*.

TRIBE V. JUNGERMANNIEÆ.

38. **Scapania** Dum. Rev. des Genres, p. 14 (1835); Hep. Eur. p. 33: G. L & N. Syn. Hep. p. 51.
**Radula,** sect. **Scapania** Dum. Syll. Jung. p. 38.
**Candollea** Raddi, in part.
**Martinellia** S. F. Gray, in part: Lindb. in Acta Soc. Fenn. x, p. 518.

Species 30–40, most numerous in northern regions; *S. undulata* and *S. nemorosa* are common and typical species.

39. **Schistocalyx** Lindb. in Jour. Linn. Soc. xiii, p. 185 (1872): Acta Soc. Sc. Fenn. x, p. 519.
**Blepharidophyllum** Ångstr. in Öfv. Vet.-Akad. Förh. xxx, p. 151 (1873).

A small subtropical genus proposed for *Scapania chloroleuca*, *S. densifolia* and their near allies.

40. **Diplophyllum** Dum. Rev. des Genres, p. 15 (1835); Hep. Eur. p. 47: Lindb. in Acta Soc. Sc. Fenn. x, p. 522: Underw. in Gray's Manual, Ed. vi, p. 715.
**Jungermannia,** sect. **Diplophyllum** Dum. Syll. Jung. p. 44.
**Jungermanniæ** sp. G. L. & N. Syn. Hep. p. 76.
**Scapaniæ** sp. Mitt. in Hook. f. Fl. Tasm. ii, p. 233.

A small genus of which the typical species is *D. albicans* Dum. As defined by Dumortier, the genus includes *Jungermannia*, sect. *Sphenolobus* Lindb.

41. **Clasmatocolea** Spruce, Hep. Amaz. et And. p. 440 (1885).

A South American genus of 2 species.

42. **Lophocolea** Dum. Rev. des Genres, p. 17 (1835); Hep. Eur. p. 83: G. L. & N. Syn. Hep. p. 151.
**Jungermannia,** sect. **Lophocolea** Dum. Syll. Jung. p. 59.

Species 50–75, especially numerous in tropical regions; *L. bidentata* and *L. heterophylla* are typical northern species.

43. **Diploscyphus** De Not. Mem. Acad. Turin (1874).

Species 1, *D. Borneensis* of Borneo.

44. **Chiloscyphus** Corda in Opiz. Naturl. p. 651 (1829): Dum. Syll. Jung. p. 67; Rev. des Genres, p. 19; Hep. Eur. p. 100: G. L. & N. Syn. Hep. p. 171.

A widely distributed genus of 30–50 species, the typical one being *C. polyanthos* Corda.

45. **Notoscyphus** Mitt. in Seemann, Fl. Vitiensis (1868).

A small genus of the Southern Hemisphere.

46. **Psiloclada** Mitt. in Hook. f. Fl. Nov. Zel. ii, p. 143 (1853); in Hook. f. Handb. N. Z. Fl. p. 518.

Species 1, *P. clandestina* of New Zealand and Tasmania.

47. **Plagiochila** Dum. Rev des Genres, p. 14 (1835); Hep. Eur. p. 42: G. L. & N. Syn. Hep. p. 22.

**Radula**, sect. **Plagiochila** Dum. Syll. Jung. p. 42.
**Candollea** Raddi, in part.
**Martinellia** S. F. Gray, in part.
**Pedinophyllum** Lindb. Bot. Not. 1874, p. 155 and in Act. Soc. Sc. Fenn. x, p. 504 (1875).

Species 125–150, very numerous in tropical regions; *P. asplenoides* is a common northern species.

48. **Mylia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 693 (1821): Lindb. in Act. Soc. Sc. Fenn. x, p. 525: Underw. in Gray's Manual, Ed. vi, p. 717.

**Jungermanniæ** sp. G. L. & N. Syn. Hep. p. 82.
**Leptoscyphus** Mitt. in Hook. Jour. of Bot. iii, p. 358 (1851), in part.
**Leioscyphus** Mitt. in Hook. f. Fl. N. Zel. ii, p. 134 (1855), in part.
**Coleochila** Dum. Hep. Eur. p. 105 (1874).

A small genus of northern regions; founded upon *Jungermannia Taylori* Hook. and the allied *J. anomala* Hook.

49. **Leptoscyphus** Mitt. in Hook. Jour. of Bot. iii, p. 358 (1851), in part.
**Leioscyphus** Mitt. in part.

A small genus occurring in tropical and south temperate regions; *L. fragilifolius* is one of Mitten's original species.

50. **Harpanthus** Nees, Eur. Leberm. ii, p. 351 (1836): G. L. & N. Syn. Hep. p. 689: Dum. Hep. Eur. p. 66.

**Pleuranthe** Tayl. in Hook. Lond. Jour. Bot. v, p. 282 (1846): G. L. & N. Syn. Hep. p. 689 (1847).

Species 2,—*H. Flotovianus* Nees and *H. scutatus* Spruce of Europe and America.

51. **Liochlæna** Nees in G. L. & N. Syn. Hep. p. 150 (1845).
**Aplozia** sp. Dum. Hep. Eur. p. 58.

Contains 2 or 3 species, the typical one being *L. lanceolata* of Europe and America.

52. **Symphyomitra** Spruce, Hep. Amaz. et And. p. 503 (1885).

A small South American genus.

53. **Jungermannia** (Rupp.) Mich. Nov. Gen. (1729): G. L. & N. Syn. Hep. p. 78. p. 73.

Widely distributed; species 150–200. In its restricted sense, the genus may be divided into the following subgenera:—*Aplozia* Dum. (*Jungermannia* proper), *Lophozia* Dum., *Anastrophyllum* Spruce, *Sphenolobus* Lindb. and *Gymnocolea* Dum. The name "Jungermannia" was first proposed by Ruppius, but Lindberg states that none of Ruppius' original species are now retained in the genus.

54. **Syzygiella** Spruce, Jour. of Bot. xiv (1876); Hep. Amaz. et And. p. 499.

A genus of tropical America, species 5 or 6.

55. **Temnoma** Mitt. in Hook. f. Handb. N. Z. Fl. p. 753 (1867).

A genus of a few mostly southern species, founded upon *Jungermannia pulchella* and *J. quadrifida.*

56. **Gymnoscyphus** Corda in Sturm. deuts. krypt. fasc. 25, p. 158: Dum. Rev. des Genres, p. 16; Hep. Eur. p. 112: G. L. & N. Syn. Hep. p. 191.

Species 1, *G. repens* of Europe.

TRIBE VI. CŒLOCAULEÆ.

57. **Schistochila** Dum Rev. des Genres, p. 15 (1835).
**Gottschea** Nees in G. L. & N. Syn. Hep. p. 13 (1844).

A beautiful genus, mostly confined to the Southern Hemisphere; species 30–40.

58. **Marsupella** Dum. Comm. bot. p. 114 (1822); Rev. des Genres, p 23; Hep. Eur. p. 125: Spruce, Revue Bryol. viii, p. 89: Underw. in Gray's Manual, Ed. vi, p. 721.
**Sarcoscyphus** Corda in Opiz. Naturl. p. 652 (1829): G. L. & N. Syn. Hep. p. 6.
**Marsupia** Dum. Syll. Jung. p. 77.
**Nardia** S. F. Gray, in part.

A small genus of northern regions; *M. emarginata* and *M. sphacelata* are widely distributed species.

59. **Southbya** Spruce in Trans. Bot. Soc. Edin. iii, p. 197 (1850): Dum. Hep. Eur. p. 133.

A northern genus of 3–5 species.

60. **Arnellia** Lindb. Kongl. Svenska Vet-Akad. xxiii, No. 5 (1889).

Species 1, *A. Fennica* of northern regions.

61. **Nardia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 694 (1821): Spruce, Revue Bryol. viii, p. 89: Underw. in Gray's Manual, Ed. vi, p. 721.

**Mesophylla** Dum. Comm. bot. p. 112 (1822); Syll. Jung. p. 80; Rev. des Genres, p. 24; Hep. Eur. p. 129.

**Alicularia** Corda in Opiz Naturl. p. 652 (1829): Dum. Syll. Jung. p. 79; Hep. Eur. p. 131: G. L. & N. Syn. Hep. p. 10.

**Solenostoma** Mitt. Jour. Linn. Soc. viii, p. 51.

**Jungermanniæ** sp. G L. & N. Syn. Hep. et Auctt.

**Aploziæ** sp. Dum. Hep. Eur.

A widely distributed genus of 15–25 species. As restricted by Spruce, l. c., the genus may be divided into the following subgenera :—*Eunardia* Spruce, *Eucalyx* Lindb., *Apotomanthus* Spruce and *Chascostoma* Lindb.

62. **Gymnomitrium** Corda in Opiz. Naturl. p. 651 (1829): G. L. & N. Syn. Hep. p. 2.

**Cesia** S. F. Gray, Nat. Arr. Br. Pl i, p. 705 (1821), not **Cæsia** R. Br. (1810).

**Acolea** Dum. Syll. Jung. p. 76 (1831); Hep. Eur. p. 121.

Species 10–20, occurring in cold regions; *G. concinnatum* is a common Alpine species.

63. **Prasanthus** Lindb. Kongl. Svenska Vet.-Akad. xxiii, No. 5 (1889).

Species 1, *P. Suecicum* of northern Europe and Asia.

64. **Dichiton** Mont. Sylloge Crypt. p. 52 (1856).

Species 1, *D. perpusillum* of Algeria.

## TRIBE VII. ACROBOLBEÆ.

65. **Lindigina** Gottsche, Ann. d Sc. Nat. 5me série, T. i, p. 137 (1864): Mitt. Jour. Linn. Soc. xvi, p. 157.

**Lindigia** Gottsche, Mexik. Leverm. p. 216 (1863). non Hampe.

**Gymnanthe** Tayl in part: Mitt. in Hook. f. Handb. N. Z. Fl. p. 519.

**Podanthe** Tayl. in Drumm. Swan River Crypt. (1846): G. L. & N. Syn. Hep. p. 789, not **Podanthes** Haw. Syn. Pl. Succ. (1812).

**Lethocolea** Mitt. in Hook. f. Handb. N. Z. Fl. p. 753.

8 species are enumerated by Mitten, the original ones being *L. Liebmanniana* and *L. Granatensis* of Gottsche.

66. **Acrobolbus** Nees in G. L. & N. Syn. Hep. p. 5 (1844).

**Gymnanthe** Tayl. in part: G. L. & N. Syn. Hep. p. 192: Dum. Hep. Eur. p. 119.

Species 1, *A. Wilsoni* Nees of Europe and South America.

67. **Tylimanthus** Mitt. in Hook. f. Handb. N. Z. Fl. p. 753 (1867).

**Gymnanthe** Tayl. in Lehm. pug. pl. viii. p. 1 (1844), not **Gymnanthes** Sw. (1788): G. L. & N. Syn. Hep. p. 192.

A small tropical or subtropical genus, proposed by Mitten for *Gymnanthe saccata*.

68. **Balantiopsis** Mitt. l. c. p. 753.
**Gymnanthe** Tayl. in part.

Mitten gives 2 species,—*B. diplophylla* and *B. erinacea.*

69. **Marsupidium** Mitt. l. c. p. 753.
**Gymnanthe** Tayl. in part.

A small genus of the South Pacific; the original species are *M. Orvilleanum*, *M. setulosum* and *M. Knightii.*

70. **Calypogeia** (sect. A) Raddi, Jung. Etr. in Mem. Moden. xviii, p. 42 (1820): Dum. Rev. des Genres, p. 21; Hep. Eur. p. 113.
**Gongylanthus** Nees, Eur. Leberm. ii, p. 405 (1836): G. L. & N. Syn. Hep. p. 196.

A genus of 2–5 species; the European *C. ericetorum* and *C. flagellifera* are Raddi's typical species.

TRIBE VIII. FOSSOMBRONIEÆ.

71. **Scalia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 704 (1821): Carringt. Brit. Hep. p. 1.
**Mniopsis** Dum. Comm. bot. p. 114 (1822); Syll. Jung. p. 75; Rev. des Genres, p. 22; Hep. Eur. p. 120.
**Haplomitrium** Nees, Eur. Leberm. i, p. 109 (1833): G. L. & N. Syn. Hep. p. 2.

Species 3,—*S. Hookeri* of Europe, and 2 species of tropical America.

72. **Rhopalanthus** Lindb. Manip. Musc. Scand. p. 390 (1874).

Species 1, *R. mnioides* of Japan.

73. **Fossombronia** Raddi, Jung. Etr. in Mem. Moden. xviii, p. 40 (1820): Dum. Rev. des Genres, p. 11; Hep. Eur. pp. 13, 173: G. L. & N. Syn. Hep. p. 467.
**Codonia** Dum. Comm. bot. p. 111 (1822); Syll. Jung. p. 29.

Species 10–20, scattered; the typical *F. pusilla* occurs in Europe.

74. **Noteroclada** Tayl. Hep. Antarc. in Lond. Jour. Bot. 1844, p. 478.
**Androcryphia** Nees, G. L. & N. Syn. Hep. p. 470 (1846).

A small genus, mostly confined to the Southern Hemisphere.

75. **Petalophyllum** Gottsche in G. L. & N. Syn. Hep. p. 472 (1846).
**Codonia** Dum. Hep. Eur. p. 16 (1874).

Species 4–8, scattered; *P. Ralfsii* occurs in the British Isles.

76. **Calycularia** Mitt. in Journ. Linn. Soc. v, p. 122 (1861).

A small tropical genus of South Asia, etc.

77. **Calobryum** Nees in Lindl. Introd. Ed. ii, p. 414: G. L. & N. Syn. Hep. p. 507.

A very doubtful genus containing one species, *C. Blumii* of Java.

78. **Treubia** Göbel, Ann. Jard. Bot. Buitenzorg, ix (1890).

A small Javanese genus.

79. **Podomitrium** Mitt. in Hook. f. Fl. Nov. Zel. ii, p. 164 (1853); in Handb. Z. Fl. p. N. 541.

Species 1, *P. Phyllanthus* of Tasmania and New Zealand.

80. **Pallavicinia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 775 (1821): Lindb. in Act. Soc. Sc. Fenn. x, p. 540: Underw. in Gray's Manual, Ed. vi, p. 723.
**Dilæna** Dum. Comm. bot. p. 114 (1822); Rev. des Genres, p. 25; Hep. Eur. p. 136.
**Diplomitrium** Corda in Opiz. Naturl. p. 653 (1829).
**Diplolæna** Dum. Syll. Jung. p. 82 (1831).
**Cordæa** Nees in Bot. Zeit. 1833, p. 401.
**Blyttia** Endl. Gen. Pl. p. 1339 (1840): G. L. & N. Syn. Hep. p. 474.
**Steetzia** Lehm. Pl. Preiss. ii, p. 129 (1846).
**Mörckia** Gottsche in Rabenh. Hep. Eur. Exsic. n. 295.
**Mittenia** Gottsche in Ann. des Sc. Nat. 5me série, T. i, p. 177 (1864).

Species 10–15, scattered; the original *P. Lyellii* is common and widely distributed.

81. **Hymenophyton** Dum. Rev. des Genres, p. 25 (1835).
**Umbraculum** Gottsche in Mohl & Schlect. Bot. Zeit. 1861, pp. 1–3; Ann. des Sc. Nat. 5me série, T. i, p. 180 (1864).

Species 3,—*H. flabellatum* Dum. and *H. leptopodon* (Tayl.) of New Zealand, and *H. Mülleri* (Gottsche) of Australia.

82. **Symphyogyna** Mont. & Nees in Ann. des Sc. Nat. 2me série, T. v, p. 66 (1836): G. L. & N. Syn. Hep. p. 479.

Species 20–30; mostly in the Southern Hemisphere.

83. **Pellia** Raddi, Jung. Etr. in Mem. Moden. xviii, p. 45 (1820): Dum. Rev. des Genres, p. 27; Hep. Eur. p. 144: G. L. & N. Syn. Hep. p. 488.
**Scopulina** Dum. Comm. bot. p. 115; Syll. Jung. p. 87.

Species 3–5, scattered; *P. epiphylla*, the original species, is widely distributed.

84. **Blasia** Mich. Nov. Gen. p. 14 (1729): G. L. & N. Syn. Hep. p. 491: Dum. Hep. Eur. p. 134.

Species 1, *B. pusilla* Linn. of northern regions.

## TRIBE IX. MONOCLEÆ.

85. **Monoclea** Hook. Musc. Exot. t. 174 (1820): G. L. & N. Syn. Hep. p. 508.

Species 2,—*M. Forsteri* Hook. of New Zealand and *M. Gottschei* Lindb. of South America.

### TRIBE X. METZGERIEÆ.

86. **Metzgeria** Raddi, Jung. Etr. in Mem. Moden. xviii, p. 45 (1820): Dum Rev. des Genres, p. 26; Hep. Eur. p 138: G. L. & N. Syn. Hep. p. 501.
**Fasciola** Dum. Comm. bot. p. 114.
**Echinogyna** Dum. Anal. fam. p. 60; Syll. Jung. p. 83.

Lindberg enumerates 11 species in his monograph of the genus; the original *M. furcata* and *M. pubescens* are common in Europe.

### TRIBE XI. ANEUREÆ.

87. **Aneura** Dum. Comm. bot. p. 115 (1822); Syll. Jung. p. 85; Rev. des Genres, p. 26; Hep. Eur. p. 140: G. L. & N. Syn. Hep. p. 493.
**Rœmeria** Raddi, Jung. Etr. in Mem. Mod. xviii, p. 46, not **Rœmeria** Medik.
**Riccardia** S. F. Gray, Nat. Arr. Br. Pl. i, p. 683 (1821), not **Richardia** Kunth in Mém. Mus. Paris, iv, p. 430 (1818).
**Sarcomitrium** Corda in Sturm Deutschl. Fl. 2 fasc. 26 and 27, p. 119.

The genus includes the following,—

**Acrostolia** Dum. Rev. des Genres, p. 26 (**Pseudoneura** Gottsche, Mexik. Leverm.).

A widely distributed genus of 20–30 species, the typical *A. pinguis* occurring in Europe and America.

## ORDER II. ANTHOCEROTACEÆ.

88. **Dendroceros** Nees in G. L. & N. Syn. Hep. p. 579 (1846).

Species 10–15, mostly tropical.

89. **Anthoceros** Mich. Nov. Gen. p. 11 (1729): G. L. & N. Syn. Hep. p. 582.

Species 20–30, most numerous in tropical regions; *A. lævis* and *A. punctatus* are the commonest northern species.

90. **Notothylas** Sulliv. Mem. Am. Acad. n. ser. iii, p. 65 (1846): Dum. Hep Eur. p. 161.
**Carpolipum** Nees in G. L. & N. Syn. Hep. p. 591.
**Chamæceros** Milde in Nov. Act. N. C. (1856).

A small genus of Europe and North America; *N. orbicularis* Sulliv. is an original species.

## ORDER III. MARCHANTIACEÆ.

### TRIBE I. MARCHANTIEÆ.

91. **Marchantia** Marchant. f. in Act. Gall. (1713): G. L. & N. Syn. Hep. p. 521; Dum. Hep. Eur. p. 150.

Widely distributed; species 25–30; *M. polymorpha* L. is the commonest.

92. **Preissia** Corda in Opiz. Naturl. p. 647 (1829): G. L. & N. Syn. Hep. p. 521.
**Chomiocarpon** Corda, l. c. p. 647.

A small genus, mostly found in northern regions; *P. commutata* Nees is the most widely distributed species.

93. **Fimbriaria** Nees, Hor. phys. Berol. p. 44 (1820): G. L. & N. Syn. Hep. p. 555.
**Hypenantron** Corda in Opiz. Naturl. p. 648 (1829).

Species 25–30, scattered; *F. tenella* is a common American species.

94. **Conocephalus** Necker, Elem. Bot. iii, p. 344 (1790): Dum. Hep. Eur. p. 154.
**Fegatella** Raddi in Opusc. scient. d. Bot. ii, p. 356 (1818): G. L. & N. Syn. Hep. p. 546.
**Hepatica** Mich. Nov. Gen. (non Dill.).

Contains 2 species,—the cosmopolitan *C. conicus* Dum. and *C. Japonicus* (Steph.).

95. **Sandea** Lindb. Acta Soc. pro F. et Fl. Fenn. T. ii, N. 5, p. 3 (1884).

Species 1, *S. supradecomposita* Lindb. of Japan and India.

96. **Sauteria** Nees, Eur. Leberm. iv, p. 139 (1838): G. L. & N. Syn. Hep. p. 541: Lindb. Acta Soc. pro F. et Fl. Fenn. T. ii, N. 3, p. 7.

Contains the typical *S. alpina* Nees and a few doubtful species.

97. **Peltolepis** Lindb. Bot. Notis. 1877, p. 73; Acta Soc. pro F. et Fl. Fenn. T. ii, N. 3, p. 3.

Species 1, *P. grandis* of Northern Europe.

98. **Clevea** Lindb. Not. Soc. pro F. et Fl. Fenn. ix, p. 289 (1868); Acta Soc. pro F. et Fl. Fenn. T. ii, N. 3, p. 10: Dum. Hep. Eur. p. 149.
**Exormotheca** Mitt. in C. Godman's Nat. Hist. Azores, p. 325 (1870).

A small genus of which the typical species is *C. hyalina* of Europe and Greenland.

99. **Athalamia** Falconer in Trans. Linn. Soc. xx, P. 3, p. 397 (1851).

Species 1, *A pinguis.*

100. **Grimaldia** Raddi in Opusc. scient. d. Bot. ii, p. 356 (1818): G. L. & N. Syn. Hep. p. 549: Dum. Hep. Eur. p. 156.
**Duvalia** Nees in Mag. d. nat. fr. zu Berl. p. 271 (1817): G. L. & N. Syn. Hep. p. 553, not **Duvalia** Haw. Syn. Pl. Succ. 414 (1812).

A small genus of Europe and America; the best known species are *G. barbifrons* and *G. rupestris.* The old genera *Grimaldia* and *Duvalia* are here united in accordance with the views of Lindberg, Underwood and others. (See Bot. Gazette, xiv, p. 197.)

101. **Cryptomitrium** Aust. in Underw. Cat. Hep. p. 36 (1884).

Species 1, *C. tenerum* of Mexico and California.

102. **Asterella** Beauv. in Encycl. meth. suppl. i, p. 502 (1810): Dum. Hep. Eur. p. 154: Underw. Cat. Hep. p. 37.

**Rebouilia** Raddi in Opusc. scient. d. Bot. ii, p. 56 (1818): G. L. & N. Syn. Hep. p. 547.

A small genus of which the typical species is *A. hemisphærica* of Europe and North America.

103. **Askepas** Griff. Notulæ, p. 341 (1849): Mitt. Jour. Linn. Soc. v, p. 127.

Species 1, *A. brevipes* of India.

104. **Dumortiera** Nees in Nov. Act. Acad. Cæs. Leop. xii, P. 1, p. 410 (1823): G. L. & N. Syn. Hep. p. 542.

Species 5–10, scattered; the typical *D. hirsuta* is found in both Europe and America.

105. **Rhacotheca** Bischoff in Hochst. & Scub. Fl. Azor. p. 12, t. 14 (1846): G. L. & N. Syn. Hep. p. 573.

Species 1, *R. Azorica.*

TRIBE II. LUNULARIEÆ.

106. **Lunularia** Mich. Nov. Gen. p. 4 (1729): G. L. & N. Syn. Hep. p. 510.

Species 1, the common *L. vulgaris.*

107. **Aitonia** Forster, Char. gen. pl. p. 147, n. 74 (1776): Lindb. in Act. Soc. Sc. Fenn. x.

**Otiona** Corda in Opiz. Naturl. i, p. 648 (1829): Dum. Hep. Eur. p. 148.

**Plagiochasma** Lehm. et Lindenb. in Lehm. Pug. pl. iv, p. 13: G. L. & N. Syn. Hep. p. 511.

Species 15–20, mostly in warm regions.

TRIBE III. TARGIONIEÆ.

108. **Targionia** Mich. Nov. Gen. p. 3 (1729): G. L. & N. Syn. Hep. p. 574.

A small genus, most frequent in warm countries; the original *T. hypophylla* occurs in Europe and America.

109. **Cyathodium** Kunze in Lehm. Pug. pl. vi, p. 17 (1834): G. L. & N. Syn. Hep. p. 577.

**Synhymenium** Griff. Notulæ, p. 344 (1849): Mitt. in Jour. Linn. Soc. v, p 124. (See Stephani in Hedwigia, xxvii, p. 250.)

**Monoselenium** Griff. l. c. p. 341: Mitt. l. c. p. 127.

Species 1, the tropical *C. cavernosum.*

## ORDER IV. RICCIACEÆ.

### TRIBE I. RICCIEÆ.

110. **Boschia** Mont. Ann. des Sc. Nat. 4me série, T. v, p. 351 (1856).

Species 1, *B Weddellii* of Brazil.

111. **Riccia** Mich. Nov. Gen. p. 107 (1729): G. L. & N. Syn. Hep. p. 598.

Widely distributed; species 50–60. The genus may be divided into the subgenera *Lichenodes*, *Spongodes*, *Ricciella* and *Ricciocarpus*, the last two of which are considered distinct genera by Dumortier and others.

112. **Tessellina** Dum. Comm. bot. p. 78 (1822): Hep. Eur. p. 164.

**Oxymitra** Bisch. in Lindenb. Syn. Hep. Eur. p. 124 (1829): G. L. & N. Syn. Hep p. 597.

**Rupinia** Corda in Opiz. Naturl. p. 650 (1829).

**Pycnoscenus** Lindb. in Öfv. Vet.-Akad. Förh. xix, p. 606 (1862).

Species 1, *T. pyramidata* of Europe, etc.

113. **Corsinia** Raddi in Opusc. scient. d. Bot. (1818): G. L. &. N. Syn. Hep. p. 596.

Species 1, *C. marchantioides* of Europe.

114. **Myriorrhynchus** Lindb. Acta Soc. pro F. et Fl. Fenn. T. ii, N. 5, p. 7 (1884).

Species 1, *M. fimbriatus* Lindb. (*Riccia fimbriata* Nees) of South America.

### TRIBE II. SPHÆROCARPEÆ.

115. **Riella** Mont. Sylloge Crypt. p. 94 (1856): Dum. Hep. Eur. p. 163.

**Duriæa** Bory & Mont. Compte. rendu. des séances de l'Acad. des Sciences (1843) G. L. & N. Syn. Hep. p. 593.

A small genus of Europe and Northern Africa.

116. **Sphærocarpus** Mich. Nov. Gen. p. 4 (1729): G. L. & N. Syn. Hep p. 594

A small genus of Europe and North America; *S. terrestris* is the most widely distributed species.

117. **Thallocarpus** Lindb. in Bull. Torr. Bot. Club, vi, p. 21 (1875): Underw Cat. Hep. p. 29.

**Cryptocarpus** Aust. in Proc. Phil. Acad. Dec. 1869, p. 231.

Species 1, *T. Curtisii* Aust. of the Southern United States.

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## XVII.—On the Ferments contained in the Juice of the Pineapple (*Ananassa sativa*), together with some Observations on the Composition and Proteolytic action of the Juice. By R. H. Chittenden, assisted by E. P. Joslin and F. S. Meara.

Some time ago the writer's attention was called to the fact that Señor V. Marcano,* of Venezuela, had discovered the existence of a proteid-digesting principle in plants of the order Bromeliaceæ, of which the pineapple is a well known representative, and that the juice of the latter fruit was being made use of as a digestive agent in the preparation of pre-digested foods.† So far as the writer is aware, there is no scientific record of this discovery other than in a short note contained in a recent number of a pharmaceutical journal,‡ in which attention is simply called to Marcano's discovery and the name "*bromelin*" suggested as an appropriate title for the hypothetical ferment.§ Apparently, no study has been made of the nature of the ferment presumably present in the juice, its mode of action, or the character of the products resulting from such action.

From a physiological standpoint the discovery of any ferment, either in the vegetable or animal kingdom, is a matter of considerable importance, especially so in the plant kingdom, since the feeling is widely gaining ground that proteid-splitting ferments must play an important part in rendering the food material of plants available. As in the animal kingdom, proteid food to be available for the needs of the plant must be transformed into soluble forms fitted for absorption and circulation. Hitherto, the best known illustration of such a vegetable proteolytic ferment has been *papaïn*, present in the juice of the papaw plant, but in the discovery of the proteolytic action of pineapple juice we have what promises to be an equally prominent illustration and one, moreover, which constitutes an addi-

* Recently deceased.

† By the Mosquera-Julia Food Co.

‡ Bulletin of Pharmacy, vol. v, p. 77, 1891.

§ Since the above was written the writer's attention has been called to the following reference contained in the Botanisches Centralblatt, No. 44, 1891: "E. Kayser, Note sur les ferments de L'ananas, Annales de l'Institute Pasteur, 1891, No. 7." To how great an extent this communication treats of the work about to be described the writer has at present no means of judging, as the above periodical is not at hand.

tional reason for believing in the probable wide-spread distribution of proteid-digesting principles throughout the vegetable kingdom.

The proteid-digesting power* of fresh pineapple juice is something quite remarkable in its intensity; it is moreover a constant feature and one which admits of easy demonstration. During the past few months great numbers of ripe pineapples have been examined in the writer's laboratory and in no instance has the juice failed to show marked proteolytic power, as evidenced by its ready solvent action on blood fibrin and other forms of proteid matter.

In addition to this proteid-digesting power, we have discovered that the juice also possesses in a remarkable degree the power of curdling or clotting milk. Neutralized pineapple juice added to milk warmed at 40° C., quickly brings about a separation of the casein, in the form of a thick clot or curd, the action being apparently exactly analogous to that of the rennet-ferment or rennin. Boiling the neutralized juice prior to its addition to the milk prevents this separation of a clot, and hence the action in question must be due to the presence of a rennet-like ferment. This ferment, indeed, we have been able to separate from the juice, together with the proteolytic ferment, by saturation of the fluid with ammonium sulphate and with this preparation we have substantiated its milk-curdling properties.

### *General character of pineapple juice.*

As is well known, the pineapple is an exceedingly juicy fruit, an average sized one of 1100 grams yielding, after chopping the tissue and subjecting it to sufficient pressure, 600–800 cubic centimeters, or considerably more than half its weight, of juice. As it flows from the press the fluid has a somewhat turbid appearance, not easily removed by filtration through paper, but eventually, as the pores of the paper become somewhat filled up, a perfectly clear yellowish colored filtrate is obtained, of very decided acid reaction and with an average specific gravity of 1043. The acidity is very pronounced, but naturally quite variable, being dependent in part upon the ripeness of the fruit. A determination of the acidity of twenty distinct samples of filtered juice showed an average acidity equivalent to 0·45 per cent. hydrochloric acid (HCl), the extremes being 0·28 per cent. and 0·65 per cent., calculated as HCl. The content of proteid matter in the clear filtered juice is quite small. Heated with Millon's reagent, a fairly strong proteid reaction is

* This was referred to by the writer in a paper read before the Philadelphia County Medical Society, May 13, 1891, an abstract of which was published in the Medical News, vol. lviii, p. 719.

obtained, the intensity of which, however, appears to be due in part to the presence of tyrosin or some related soluble body.

With acetic acid alone no precipitate is produced, but with acetic acid and potassium ferrocyanide a slight precipitate or turbidity results. With the biuret test, the reaction is in great part vitiated by the large amount of sugar present in the juice, which gives rise to an intense yellowish-brown color on addition of the strong alkali.

Neutralization of the acid juice with sodium carbonate fails to give any neutralization precipitate whatever, thus showing the absence of acid-albumin. Concentrated nitric acid produces in the clear filtered juice a white precipitate soluble in excess of the acid, the fluid taking on a bright yellow color.

Fresh pineapple juice filtered clear and with average acidity, subjected to fractional heat precipitation grows slightly turbid at 60–62° C., the turbidity increasing gradually as the temperature is raised until 75–78° C. is reached, when a small flocky coagulum results. The filtrate from this coagulum on being further heated shows signs of turbidity at about 82° C., increasing with the rise in temperature, without however any distinct signs of flocking until the boiling point is reached. As the fluid commences to boil, but sometimes only after persistent boiling, a fine flocky precipitate separates, which on filtration leaves a perfectly clear fluid. This fluid, free from all matter coagulable by heat, gives with Millon's reagent the usual proteid reaction, while with acetic acid and potassium ferrocyanide, it yields a distinct white precipitate. Concentrated nitric acid alone gives no reaction, but addition of saturated-salt solution with the acid causes a distinct turbidity, which is increased rather than diminished by heat. We have thus evidence of the presence in pineapple juice of what appears to be three distinct proteids; two separable from the acid juice by heat alone, one at about 75° C., the other at 100° C., while the third body is wholly non-coagulable by heat, but is precipitable by acetic acid and potassium ferrocyanide This latter proteid can also be precipitated by saturation of its solution with ammonium sulphate (after removal of the proteids separable by boiling) together with some non-proteid matter present in the juice, and after removal of the ammonium sulphate by dialysis gives the reactions above indicated. It is present only in very small quantity. In some cases, however, this third body is present in the juice in larger quantity, or to express it more exactly, in some specimens the precipitate produced by acetic acid and potassium ferrocyanide in the filtrate from the heat precipitations is considerably more pronounced than first described.

The two precipitates produced by heat alone, viz: at 75–78° C. and at 100° C. are not coagulated proteids in the ordinary sense of the term. Unlike an ordinary coagulum of albumin or globulin, these precipitates, when filtered off and washed with water, dissolve readily and almost entirely in dilute solutions of potassium hydroxide; they are also more or less completely soluble in 0·5 per cent. solution of sodium carbonate, especially if warmed, and are somewhat soluble in 0·2 per cent. hydrochloric acid. In strong nitric acid on the other hand, both precipitates are insoluble unless a large quantity of the acid is added. They are likewise insoluble in 10 per cent. solution of sodium chloride. The proteid separable by heat at about 75° C. appears somewhat more soluble in 0·5 per cent. sodium carbonate than the substance separating at 100° C., the latter dissolving completely in 0·5 per cent. sodium carbonate only when the mixture is heated to boiling. The solution of the above precipitates in dilute alkali is not alkali-albumin, although the substance is in part precipitated by neutralization of the alkaline fluid, since nitric acid gives a precipitate apparently wholly insoluble in excess of the acid, even on heating.

These two proteids, when dissolved in potassium hydroxide and tested with a few drops of a dilute solution of cupric sulphate, give a violet rather than a red biuret reaction.

While thus these two proteids precipitated by heat, at 75° and 100° C. respectively, are not exactly akin to an ordinary albumin or globulin in their behavior towards heat, neither do they closely resemble on the other hand an ordinary albumose, like the $\beta$ phytalbumose of Martin* present in papaw juice, since the precipitates are not readily soluble in nitric acid, even when heated, and when once dissolved are not separable on cooling the solution.

As already stated, the amount of proteid matter in pineapple juice is comparatively small and apparently these two proteids, separable by boiling, compose the greater part of the albuminous matter. The amount was determined by simply heating 100$^{cc}$ of filtered (acid) juice to boiling, collecting the precipitate on a weighed filter, washing it thoroughly with boiling water and drying it at 110° C. until of constant weight. The result showed the presence of only 0·0270 gram in the 100$^{cc}$ of juice, or less than 0·025 per cent.

When *neutralized* pineapple juice is subjected to heat precipitation, the initial turbidity makes its appearance at about 72°–74° C. with separation of flocks at 82°–83° C. The filtrate from this pre-

* Journal of Physiology, vol. vi, p. 347.

cipitate remains clear even when the solution is boiled, but a drop or two of acetic acid added to the hot fluid produces a turbidity, which on further heating eventually changes to a flocculent precipitate. In the filtrate from this second precipitate, acetic acid and potassium ferrocyanide show the presence, in small quantity, of what is presumably a non-coagulable proteose. It is thus evident that the presence of acid lowers the temperature at which these bodies are precipitated by heat, and further that the substance precipitated at 100° C. can be made to separate only in the presence of dilute acid. This fact certainly favors the view that the juice contains two distinct proteids precipitable by heat, the one at about 75° C. in an acid solution or 82° C. in a neutral fluid, the other at 100° C. in an acid fluid. On the other hand, the above reactions might be produced by a single substance slowly or incompletely precipitated by heat, analogous to the separation of Martin's* $\beta$ phytalbumose, which is described as separating in two distinct stages, viz: at from 78°–82° C., and from 83°–95° C. As against this latter view, however, we have the apparent fact that in the pineapple juice, one of the proteids is precipitated from a neutral or acid fluid by heat, the other only from an acid fluid.

Long continued dialysis (10–12 days) of neutralized pineapple juice in running water, protected from putrefaction by addition of thymol, gives little or no separation of any proteid matter. A faint turbidity may appear, but no separation sufficiently large to collect on a filter. Such turbidity as does make its appearance clears up on the addition of a few drops of a strong solution of sodium chloride, or of dilute nitric acid; two reactions equally characteristic of a globulin or of heteroproteose.

Saturation of neutralized, or acid, pineapple juice with pure ammonium sulphate precipitates all of the proteids present in the fluid, together with a small amount of non-albuminous matter. In this precipitate are contained both the proteolytic and rennet-like ferments.

Saturation of neutralized pineapple juice with sodium chloride gives rise to a small flocculent precipitate of proteid matter, which is not at all increased by the addition of acetic acid to the salt-saturated fluid. Addition of ammonium sulphate in substance to the filtrate from the salt-saturation precipitate produces a further precipitate of proteid matter, thus suggesting a possible separation of two distinct bodies. Further, simple boiling of the filtrate from

* Journal of Physiology, vol. vi, p. 349.

the sodium chloride saturation produces a flocky precipitate, substantiating this view.

Saturation of the neutralized juice with magnesium sulphate likewise produces a precipitate of proteid matter, somewhat heavier than that induced by saturation with sodium chloride. Addition of crystals of sodium sulphate to the filtrate from the above precipitates causes finally a slight additional separation of flocculent matter containing a little proteid.

Both the sodium chloride precipitate and the magnesium sulphate precipitate are strongly proteolytic.

The general character of these precipitates will be discussed more in detail later on, in connection with the isolation of the proteolytic ferment.

*Proteolytic action of fresh pineapple juice under varying conditions.*

As already stated, fresh pineapple juice is strongly proteolytic, and its proteid-digesting power is manifested in a neutral, acid, or even alkaline-reacting fluid. In this respect, therefore, the ferment resembles trypsin rather than pepsin.

When blood fibrin is warmed at 40° C., or thereabout, with filtered pineapple juice of average acidity the fibrin swells up somewhat in the acid fluid, then quickly becomes disintegrated and in part dissolved, the initial action certainly being as vigorous as that of a moderately strong solution of pepsin-hydrochloric acid. There invariably remains, however, even after long-continued warming at 40° C., a fairly large insoluble residue, not of unaltered fibrin, but of finely divided antialbumid-like matter more or less soluble in weak alkaline fluids, from which it is reprecipitated by the addition of dilute acid.

Pineapple juice neutralized, or made very faintly alkaline, with dilute sodium carbonate acts apparently in much the same manner as the acid juice, except that in the alkaline-reacting fluid there is less residue of antialbumid-like matter and in the neutralized juice, naturally, an utter absence of any swelling of the fibrin. An examination of the several digestive mixtures, however, shows that the products formed in a neutral or alkaline solution are different somewhat from those formed in an acid-reacting fluid, a point which will be referred to again later on.

One of the most noticeable features in the digestive action of the pineapple ferment is its peculiar softening and disintegration of the proteid matter. This is most noticeable in a neutral solution; thus

when blood fibrin, for example, is warmed with neutralized pineapple juice, or better, with a neutral solution of the isolated ferment there is at first no sign of any digestive action whatever, but on stirring or shaking the mixture, after a sufficient length of time, the fibrin falls to pieces completely disintegrated with the production of a more or less turbid fluid, after which its solution is fairly rapid, although there invariably remains considerable insoluble matter, the same as in an acid mixture. While this action of the ferment resembles somewhat that of trypsin, there is never seen that peculiar eating into the fibrin, so characteristic of the latter ferment; the fibrin never has the appearance of being full of tiny holes, as if bored by a host of worms, so often seen in a trypsin digestion. The pineapple ferment appears simply to soften the fibrin with more or less solvent action at the same time, so that when stirred or pressed it breaks apart into larger or smaller pieces, these in turn undergoing a like change until the fibrin is thoroughly disintegrated and the soluble portion dissolved.

### 1.—*Influence of the reaction of the fluid.*

As is well known, trypsin* and papaïn† act best in an alkaline medium; the pineapple ferment on the other hand acts most energetically in a neutral solution, although the ferment is decidedly active in the presence of both acids and alkali carbonate.

In studying the effect of changes in the reaction of pineapple juice on its proteolytic power, or in measuring the proteolytic action of the ferment under varying conditions, the following method was, as a rule, made use of; a given volume of filtered pineapple juice, usually 100 c. c., was warmed at 40° C. for a given length of time with 10 grams of moist, freshly coagulated egg-albumin, which had been completely freed from all soluble matter by thorough washing with hot water. When the period of digestion was completed the undissolved matter was collected on a weighed filter, washed with water until all soluble bodies were removed and then dried at 110° C. until of constant weight. By subtracting the weight of the insoluble residue so obtained from the weight of dry albumin‡ equivalent to the moist albumin used in the experiment, the amount of proteid matter digested, or rather converted into soluble products, was ascertained. Obviously, however, the so-called undissolved portion of

* Studies from the Laboratory of Physiological Chem., Yale University, vol. i, p. 135.

† Martin, Journal of Physiology, vol. v, p. 221; vol. vi, p. 336.

‡ Determined by simply drying 10 grams of the sampled coagulated albumin at 110° C until of constant weight.

the albumin was in part composed of insoluble antialbumid-like matter, especially when the digestions were carried on in acid or neutral media. The method, however, afforded a fairly accurate means of measuring the proteolytic power of the ferment, as contained in pineapple juice, while the greater resistance of coagulated albumin as compared with blood fibrin seemed to offer advantages in the way of accuracy.

*Experiment I.*—The pineapple juice employed had an acidity equal to 0·445 per cent. HCl,* requiring 13 c. c. of a 5·0 per cent. solution of $Na_2CO_3$ to neutralize 100 c. c. The 10 grams of moist coagulum used in each digestive mixture contained 1·3633 grams of dry albumin (at 110° C.). The mixtures were warmed at 40° C. for 3½ hours.

| | Pineapple juice. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c.† | natural acidity | 0·8932 gram | 34·5 |
| B | 100 | neutralized | 0·8187 | 40·0 |

*Experiment II.*—The acidity of the pineapple juice employed was equal to 0·507 per cent. HCl. Necessary to neutralize 100 c. c., 14·7 c. c. of a 5·0 per cent. solution of $Na_2CO_3$. Weight of dry albumin equivalent to the 10 grams of moist coagulum used in each mixture, 1·3302 grams. The digestions were carried on at 40° C. for 2 hours.

| | Pineapple juice. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | natural acidity | 1·0586 grams | 20·8 |
| B | 100 | neutralized | 0·9355 | 29·7 |

*Experiment III.*—The acidity of the pineapple juice was not accurately determined. 15 c. c. of a dilute solution of sodium carbonate were required to neutralize 100 c. c. of juice. The weight of dry albumin contained in the 10 grams of moist coagulum used in the digestions was 1·4583 grams. The mixtures were warmed at 40° C. for 5½ hours.

| | Pineapple juice. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | natural acidity | 0·8926 gram | 38·8 |
| B | 100 | half-neutralized | 0·8733 | 40·2 |
| C | 100 | neutralized | 0·8115 | 44·4 |

From these results, it is seen that full 40 per cent. or more of coagulated egg-albumin can be converted into soluble products by the pineapple ferment, under the conditions of the above experi-

* Determined by titration with a standard solution of ammonium hydroxide.

† Plus the amount of sodium carbonate solution required for neutralization in B, and of water to make an equal dilution in A.

ments, and further that the neutralized juice is considerably more active than the unneutralized fluid. Apparently, the proteolytic action of the ferment increases with the decrease in acidity, until the neutral point is reached.

With blood fibrin, on the other hand, juice of the natural acidity appears to have a greater digestive power than the neutralized fluid, although on this point we have only a single experiment to offer. This may perhaps be explained simply by the swelling of the fibrin in the dilute organic acid, this condition possibly facilitating the action of the ferment.

*Experiment IV.*—The acidity of the pineapple juice was equal to 0·525 per cent. HCl. Necessary to neutralize 100 c. c. of filtered juice, 15·2 c. c. 5·0 per cent. solution of $Na_2CO_3$. Weight of dry albumin contained in 10 grams of moist coagulum, 1·4486 grams. Weight of dry fibrin (at 110° C.) contained in 6 grams of washed blood fibrin,* the amount used in the digestions, 2·5273 grams. The digestive mixtures were warmed at 40° C. for 2 hours.

| | Pineapple juice. | Reaction. | Undissolved proteid. | Per cent. digested. |
|---|---|---|---|---|
| Blood fibrin . . . | 100 c. c. | natural acidity | 1·2435 grams | 50·8 |
| | 100 | neutralized | 1·4321 | 43·3 |
| Egg-albumin . . | 100 | natural acidity | 1·0325 | 28·8 |
| | 100 | neutralized | 1·0096 | 30·3 |

The most noticeable feature in this experiment, aside from the point already mentioned, is the far greater digestibility of blood fibrin as compared with egg-albumin, a fact which might naturally be expected, since the same is true in the case of other well-known proteolytic ferments.

The proteids of muscle tissue are likewise more readily digested by pineapple juice than coagulated egg-albumin, full 60 per cent. of the former proteids being converted into soluble products during one hour's warming at 40° C. This is plainly shown in the following experiment, in which also the muscle proteids, like egg-albumin, are seen to be more rapidly digested in the neutralized juice than in the acid fluid.

*Experiment V.*—The acidity of the pineapple juice employed was equal to 0·507 per cent. HCl. Necessary to neutralize 100 c. c. of juice, 14·7 c. c. of a 5 per cent. solution of $Na_2CO_3$. Weight of dry

* Washed with water and salt solution, then boiled in water, alcohol, and lastly in water.

proteids contained in 10 grams of prepared muscle* tissue, 2·7258 grams.

| | Pineapple juice. | Reaction. | Undissolved proteid. | Per cent. digested. |
|---|---|---|---|---|
| ½ hour at 40° C. | 100 c. c. | natural acidity | 1·8945 grams | 30·6 |
| | 100 | neutralized | 1·1366 | 58·4 |
| 1 hour at 40° C. | 100 | natural acidity | 1·6368 | 40·1 |
| | 100 | neutralized | 1·0145 | 62·8 |

The acidity of pineapple juice is due to organic acids and acid salts, far weaker in their action on ferments than mineral acids. Addition of dilute mineral acid in small quantity to pineapple juice of natural acidity checks, but does not prevent the digestive action of the ferment. Thus, the addition of an equal volume of 0·2 per cent. hydrochloric acid to pineapple juice diminishes very greatly its digestive power, but does not prevent it altogether. From this we may conclude that pineapple juice can exert its proteolytic power, to a certain extent, when taken into the stomach and mixed with the acid of the gastric juice. Obviously, the addition of an equal volume of 0·2 per cent. hydrochloric acid to neutralized pineapple juice does not necessarily mean the presence of 0·1 per cent. HCl, since the acid may be in great part used up in decomposing the various salts present, and in combining with the various forms of organic matter contained in the juice.

*Experiment VI.*—The acidity of the pineapple juice employed was equal to 0·288 per cent. HCl. The 10 grams of moist albumin coagulum used in the digestions contained 1·7972 grams of dry albumin. The mixtures were warmed at 40° C. for 17 hours.

| | Pineapple juice of natural acidity. | | | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|---|
| A | 100 c. c. + 100 c. c. $H_2O$ | | | 1·2238 grams | 32·0 |
| B | 100 | + 100 | 0·2 % HCl | 1·4673 | 19·0 |

Were it not for the large quantities of salts, etc., in pineapple juice the above inhibitory action of the hydrochloric acid on the ferment would be far more pronounced. This is shown by the two following experiments :

By adding about five volumes of 95 per cent. alcohol to pineapple juice, a flocculent precipitate results composed of the proteids of the juice, together with the proteolytic ferment and some salts. On dissolving this precipitate in water a solution is obtained with marked proteolytic power.

* Prepared by soaking chopped muscle tissue, freed from fat and tendons, in water until all blood and soluble extractives were removed.

*Experiment VII.*—Aqueous solution of the above described alcoholic precipitate. The 10 grams of moist albumin coagulum used in the digestions contained 1·4138 grams of dry albumin. The mixtures were warmed at 40° C. for 4 hours.

| | Ferment solution. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | $100^{cc} + 100^{cc}$ $H_2O$ | neutral | 1·0881 grams | 23·1 |
| B | 100 + 100 0·2 % HCl | 0·1 % HCl | 1·4099 | 0·3 |

Thus, with this very impure preparation of the ferment the presence of 0·1 per cent. hydrochloric acid was sufficient to entirely prevent any digestive action whatever. Doubtless, even smaller amounts of acid would have the same influence on the more perfectly isolated ferment.

As already stated, saturation of pineapple juice, either neutralized or of natural acidity, with ammonium sulphate precipitates all or nearly all of the proteids present in the fluid, the precipitate showing by its proteolytic action that it contains the ferment as well. An aqueous solution of such a precipitate, dialyzed to free it from ammonium sulphate, has a marked digestive action, but when mixed with hydrochloric acid its proteolytic power, like that of the alcohol precipitate, is immediately checked.

*Experiment VIII.*—Aqueous, dialyzed solution of the above described ammonium sulphate precipitate. The amount of dry albumin equivalent to the 10 grams of moist coagulum used in the digestions was 1·5120 grams. The mixtures were warmed at 40° C. for 5 hours.

| | Ferment solution. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | $100^{cc} + 100^{cc}$ $H_2O$ | neutral | 1·1791 grams | 22·1 |
| B | 100 + 100 0·2 % HCl | 0·1 % HCl | 1·4897 | 1·5 |

As previously stated, pineapple juice, and the isolated ferment as as well, manifests its proteolytic action in an alkaline-reacting fluid, as well as in the presence of an acid or neutral reaction. When, however, the solution becomes strongly alkaline proteolytic action is quickly retarded, the ferment in this respect differing very decidedly from the related ferments papaïn and trypsin. Thus, the addition of small quantities of sodium carbonate to neutralized pineapple juice, a few hundredths of one per cent., produces no noticeable effect, but as the quantity is increased the retarding action of the alkali becomes more pronounced, until at last it checks the proteolytic action of the ferment altogether. This is clearly shown in the following experiments:

*Experiment IX.*—The acidity of the pineapple juice employed was equal to 0·462 per cent. HCl. Necessary to neutralize 100$^{cc}$ of juice, 13·4$^{cc}$ of 5·0 per cent. solution of $Na_2CO_3$. The 10 grams of moist albumin coagulum used in the digestions contained 1·3516 grams of dry albumin. The mixtures were warmed at 40° C. for 1½ hours.

| | Pineapple juice. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100$^{cc}$ | ¾ neutralized | 0·9663 gram | 28·6 |
| B | 100 | neutralized | 0·9465 | 30·0 |
| C | 100 | 0·025 % $Na_2CO_3$ | 0·9522 | 29·6 |
| D | 100 | 0·05 " | 0·9735 | 28·0 |
| E | 100 | 0·10 " | 0·9968 | 26·3 |

*Experiment X.*—The acidity of the pineapple juice employed was equal to 0·656 per cent. HCl. Necessary to neutralize 100$^{cc}$, 19·1$^{cc}$ of 5·0 per cent. solution of $Na_2CO_3$. The amount of dry albumin equivalent to the 10 grams of moist coagulum used in the digestions was 1·3468 grams. The mixtures were warmed at 40° C. for 2 hours.

| | Pineapple juice. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100$^{cc}$ | neutralized | 1·0257 grams | 23·9 |
| B | 100 | 0·1 % $Na_2CO_3$ | 1·0577 | 21·5 |
| C | 100 | 0·5 " | 1·2268 | 9·0 |
| D | 100 | 1·0 " | 1·3520 | 0 |

Hence, as is evident from the above experiments, the addition of sodium carbonate to neutralized pineapple juice to the extent of 0·5 per cent., almost completely stops the action of the ferment, while the presence of 1·0 per cent. of the alkali carbonate checks it altogether. Doubtless, the isolated ferment would show a still greater susceptibility to the action of dilute alkaline fluids.

From the foregoing, it is evident that digestion with bromelin, the ferment of pineapple juice, goes on most vigorously in neutral solutions, but that the presence of small amounts of acid, especially such as are contained in pineapple juice, and of sodium carbonate interfere with the proteolytic action only slightly; larger amounts, however, check the action of the ferment altogether.

It is further evident from the foregoing results that the proteolytic ferment of pineapple juice is an exceedingly vigorous ferment. We cannot say definitely how much pure ferment by weight is contained in 100$^{cc}$ of filtered pineapple juice. There is no doubt that the amount varies greatly in different specimens of fruit; in fact, our results show plainly differences in proteolytic power hard to be accounted for in any other way. Experiments to be described

later show that the proteolytic ferment is either precipitable by heat, or else is associated with proteid bodies so precipitated. Now since the total amount of matter precipitable by boiling from 100$^{cc}$ of filtered pineapple juice amounts to only 27 milligrams, and this obviously cannot be all proteolytic ferment, it is probable that the amount of pure ferment contained in the quantity of pineapple juice used in the various digestions recorded does not amount to more than a few milligrams, and yet in one experiment with the above quantity of ferment the equivalent of 1714 milligrams of dry muscle proteids were dissolved in one hour at 40° C., and of blood fibrin an amount equivalent to 1283 milligrams of dry proteid in two hours at 40° C.

With such vigorous digestive action as this, many possibilities suggest themselves in the way of practical application of the isolated ferment, or even of the pineapple juice itself. As a means of peptonizing foods it offers peculiar advantages in that the products of digestion, to be referred to later, are free from the objectionable taste usually associated with peptones resulting from the proteolytic action of animal ferments. Again, the ferment cannot but constitute a good solvent for pseudo-membranes, while its vegetable origin would perhaps recommend it as a more agreeable remedy than the kindred ferments from animal tissue. In some sections, popular opinion has already accredited to pineapple juice virtue as a solvent for the false membranes formed in diphtheria, a belief which is now seen to be founded on a reliable basis.

### 2.—*Influence of temperature.*

It is a matter of common observation that the digestive ferments, or enzymes, present in the animal organism act most energetically at approximately the body temperature, viz: 38°–40° C. Certain of the vegetable ferments on the other hand, notably the diastase of malt, act most vigorously at a higher temperature. With papaïn, the proteolytic ferment of papaw juice, Martin demonstrated the greater activity of the ferment at temperatures between 30° and 36° C. than between 18° and 20° C. in neutral solutions,* but apparently the effect of higher temperatures was not tried.

*Experiment XI.*—The 10 grams of moist albumin coagulum used in the digestions contained 1·4990 grams of dry albumin. The several mixtures were warmed with the albumin at the stated tem-

* Journal of Physiology, vol. v, p. 221.

peratures for 2¼ hours, the juice having been first brought to the desired temperature prior to the addition of the albumin.

| | Neutralized pineapple juice. | Temperature. | Undissolved albumin | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | 12° C. | 1·3090 grams | 12·7 |
| B | 100 | 20 | 1·3037 | 13·1 |
| C | 100 | 40 | 1·2281 | 18·1 |
| D | 100 | 49 | 1·1959 | 20·3 |
| E | 100 | 56 | 1·1709 | 21·9 |

Although in this experiment, the proteolytic action of the juice, for some reason, was not as great as usual the results show in a general way that the activity of the ferment increases with the rise in temperature up to 56° C. Further, that the ferment is active at comparatively low temperatures; although there is a striking difference (nearly 50 per cent.) in activity between the two extremes, viz: at 12° and 56° C. That this peculiar ferment is truly more active at 50°–60° C. than at 30°–40° C., under the above conditions, is confirmed by the two following experiments:

*Experiment XII.*—The weight of dry albumin equivalent to the 10 grams of moist coagulum used in each digestion was 1·2937 grams. The several portions of neutralized pineapple juice were brought to the required temperatures in carefully regulated water-baths, and when the desired point was reached *the albumin was at once added* and the mixtures kept at the stated temperatures for two hours, after which, as in the other experiments, the undissolved albumin was filtered off, washed, dried and weighed.

| | Neutralized pineapple juice. | Temperature. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | 40° C. | 1·0259 grams | 20·7 |
| B | 100 | 49 | 0·9648 | 25·5 |
| C | 100 | 58 | 0·9337 | 27·8 |
| D | 100 | 66 | 0·9721 | 24·9 |

*Experiment XIII.*—This experiment was conducted in essentially the same manner as the preceding, but at different temperatures. The weight of dry albumin equivalent to the 10 grams of moist coagulum used in the individual digestions was 1·3710 grams. The ferment was allowed to act on the albumin for two hours at the respective temperatures. In this experiment, duplicate digestions were made and the results are interesting as showing about how much variation may be expected from the errors naturally incidental to methods of this character.

| | Neutralized pineapple juice. | Temperature. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | 50° C. | 0·9190 gram | 33·0 |
| B | 100 | 60 | 0·9289 | 32·3 |
| C | 100 | 60 | 0·9286 | 32·3 |
| D | 100 | 70 | 1·0669 | 22·2 |
| E | 100 | 70 | 1·0562 | 23·0 |
| F | 100 | 80 | 1·3665 | 0·4 |

From these two experiments it is plain that the ferment as contained in *neutralized* pineapple juice is most active, on coagulated egg-albumin at least, between the temperatures of 50° and 60° C. and, further, that even at 70° C. the ferment is decidedly active. At 80° C. there is practically no action whatever. In this connection it is to be remembered that neutralized pineapple juice when subjected to heat precipitation grows slightly turbid at 72°–74° C., with separation of a flocky precipitate at about 82° C. Doubtless the destruction of the ferment by heat above 70° C. is associated with this precipitation, it being quite possible that it is the destroyed ferment itself which is so precipitated.

When pineapple juice of *natural acidity* is warmed with egg-albumin at the above high temperatures, a result quite different from the preceding is obtained; under such conditions, the proteolytic action of the ferment is diminished rather than increased and at 70° C. or under, the ferment is completely destroyed. This is shown in the following experiment.

*Experiment XIV.*—The pineapple juice employed had an acidity equal to 0·454 per cent. HCl. The 10 grams of moist coagulum used in the digestions contained 1·2956 grams of dry albumin. The mixtures of pineapple juice and albumin were warmed at the given temperatures for two hours, 100 c. c. of pineapple juice being used in each digestion.

| Temperature. | Reaction. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|
| 40° C. | natural acidity* | 0·9667 gram | 25·4 |
| 40 | neutral | 0·9679 | 25·3 |
| 55 | natural acidity | 1·0672 | 17·7 |
| 55 | neutral | 0·8968 | 30·8 |
| 70 | natural acidity | 1·3012 | 0 |
| 70 | neutral | 1·0581 | 18·4 |

From these results it is seen that while the neutralized fluid is more active at 55° C. than at 40° C., thus confirming the previous

* In this individual experiment, the neutralized and acid fluids, for some reason, show exactly the same digestive power at 40° C.

data, the acid-reacting fluid, on the other hand, shows far less digestive power at 55° C. than at 40° C. and further, at 70° C. is entirely devoid of digestive action, while the neutral fluid at the latter temperature is strongly proteolytic. The general trend of these results, therefore, is to show that the ferment in a neutral solution will withstand exposure to high temperatures better than in an acid-reacting fluid; and further, that while the ferment in a neutral solution, as in neutralized pineapple juice, acts most energetically between 50° and 60° C., in an acid solution proteolytic action is most vigorous in the neighborhood of 40° C.

With pepsin, Biernacke* has shown that an acid solution increases the resistance of the enzyme to the destructive action of a high temperature; thus this ferment in the presence of 0·2 per cent. hydrochloric acid may be heated up to 60° C. before it is killed, while in a neutral solution of the same strength the ferment is destroyed at 55° C. Trypsin, on the other hand, was found more resistant to heat in an alkaline fluid than in a neutral or weakly acid solution.

In this connection it is interesting to notice that the acid-reacting pineapple juice (natural acidity) subjected to heat precipitation grows turbid at 60°–62°, with separation of flocks at about 75° C.; in other words, the destruction of the ferment by heat in the acid-reacting fluid is coincident with the commencement of the precipitation, the same as in the neutralized juice.

While *neutralized* pineapple juice is extremely active on proteid matter at the high temperatures stated, exposure of the ferment solution by itself *in the absence of any proteid matter*, to the above temperatures for even a short time quickly destroys the ferment. This fact is clearly shown in the following experiment:

*Experiment XV.*—Given volumes of neutralized pineapple juice were placed in water-baths having the desired temperatures, and when the solutions themselves had reached the temperatures stated in the following table they were kept at that point for fifteen minutes, after which they were removed from the baths, cooled to 40° C. and 10 grams of coagulated egg-albumin added. The mixtures were then warmed at 40° C. for two hours and the proteolytic action determined in the usual manner. The amount of dry albumin (at 110° C.) equivalent to the 10 grams of moist coagulum used in the individual digestions was 1·3364 grams.

* Das Verhalten der Verdauungsenzyme bei Temperaturerhöhungen. Zeitschrift für Biologie, Band xxviii, p. 49.

| | Neutralized pineapple juice. | Warmed for 15 minutes at | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | 40° C. | 1·1060 grams | 17·3 |
| B | 100 | 60 | 1·2049 | 9·9 |
| C | 100 | 70 | 1·2971 | 8·0 |
| D | 100 | 80 | 1·3420 | 0 |

While neutralized pineapple juice is capable of digesting more proteid matter at 60° C. than under like conditions at 40° C., warming the ferment solution alone at 60° for fifteen minutes, prior to the introduction of the albumin, diminishes the proteolytic power of the ferment full 50 per cent. This result suggests that the products of digestion protect the ferment, to a certain extent, from the destructive action of the high temperature. The ferment acts quickly on proteid matter, so that even after a few minutes exposure of a mixture of albumin and pineapple juice to 40°–70° C., some proteoses and peptone are doubtless formed, which can in some manner exert their protective action. When, however, the neutralized juice alone is heated to 60° C., in the absence of proteoses and peptone, the ferment is rapidly destroyed. Doubtless, the ferment as contained in pineapple juice is more resistant to heat, than a solution of the isolated ferment would be, from the possible protective action of salts present, although of this point we cannot speak definitely. These results accord with Biernacki's* observations on the ferments pepsin, trypsin and ptyalin. This investigator found that albumose, amphopeptone and antipeptone raised the temperature at which trypsin was destroyed five degrees or more; that peptone raised the temperature at which pepsin was destroyed in acid solution, from 50°–55° C. up to 70° C. These results may perhaps be explained on the ground of a combination of the ferments with the albuminous bodies, the hypothetical compounds having greater resistance towards heat than the ferment alone. In any event, the general statement may be made that the purer the ferment the less resistant will it probably be to the destructive action of high temperatures.

While the preceding results show that the pineapple ferment, as contained in filtered pineapple juice, is liable to be killed on long-continued exposure to temperatures favorable for its proteolytic action, the fluid may be heated at temperatures under 40° C. for long periods of time, or even evaporated to dryness, without destruction of the ferment, provided the temperature is carefully regulated and

* Zeitschrift für Biologie, Band xxviii, p. 49.

not allowed to pass beyond 40° C. This is demonstrated by the following experiment:

*Experiment XVI.*—The pineapple juice employed had an acidity equal to 0·445 per cent. HCl, 100 c. c. requiring 13·0 c. c. of a 5·0 per cent. solution of sodium carbonate for neutralization. The 10 grams of albumin coagulum used in the digestions contained 1·3633 grams of dry albumin.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 100 c. c. | pineapple | juice | +13·0 c. c. | 5 per cent. | $Na_2CO_3$ | sol. |
| B | 100 " | " | | +13·0 " | " | " | " |
| C | 100 " | " | | +13·0 " | | $H_2O$ | |
| D | 100 " | " | | +13·0 " | | " | |

Solutions B and D were evaporated to dryness on plates at 40° C., the residues dissolved in water and made up to 113 c. c. respectively. All four solutions were then mixed with 10 grams of albumin coagulum and warmed at 40° C. for 3½ hours, after which the amount of albumin digested was determined in the usual manner.

| | Reaction, etc. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|
| A | neutral | 0·8177 gram | 40·1 |
| B | " evaporated | 1·0215 | 25·1 |
| C | natural acidity | 0·8932 | 34·5 |
| D | " evaporated | 0·8638 | 36·7 |

From these results, it is plain that pineapple juice of natural acidity can be evaporated to dryness at a temperature not exceeding 40° C. and still preserve its proteolytic power. With neutralized juice, however, the above results indicate a partial destruction of the ferment during the evaporation. Whether this is due to the action of the neutral salts formed by neutralization of the acid, or to some other cause we cannot say. Possibly, the solution may have been made slightly alkaline, which would naturally give rise to some destruction of the ferment. Several repetitions of the above experiment, less carefully conducted, have shown that evaporation of the acid juice, however, is very liable to result in a partial loss of proteolytic power, unless great care is taken in keeping the evaporating fluid at 40° C. or under.

### 3.—*Rate of action.*

Pineapple juice is not only exceedingly active on proteid matter, but under favorable circumstances the digestive power of the ferment is quickly manifested. On blood fibrin and muscle tissue, especially, the proteolytic action of the ferment is shown in a *rapid*

solution of the proteid substance; in fact, a single observation of the manner in which blood fibrin is attacked by pineapple juice is sufficient to give one a just appreciation of the energy of the ferment. Thus in Experiment IV, it will be remembered that 50 per cent. of the blood fibrin used in the experiment was converted into soluble products in two hours, and that in Experiment V, with the proteids of muscle tissue, 58 per cent. of the proteid matter was dissolved by neutral pineapple juice in half an hour at 40° C. Naturally, on coagulated egg-albumin the digestive power of the ferment is less quickly manifested, but the experiments already recorded show that even with this more difficultly digestible proteid, the rate of action is fairly rapid. The two experiments following give a general impression of the rate of action of the ferment, in the digestion of coagulated egg-albumin.

*Experiment XVII.*—The 10 grams of albumin coagulum used in the digestions contained 1·3944 grams of dry albumin. The mixtures were warmed at 45° C. for the periods stated in the following table, the amount of albumin digested during the periods being then determined in the usual manner.

| | Neutralized pineapple juice. | Time. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | ¼ hour | 1·3026 grams | 6·6 |
| B | 100 | 1 | 1·2072 | 13·5 |
| C | 100 | 2 | 1·0827 | 22·4 |
| D | 100 | 4 | 0·8814 | 36·8 |
| E | 100 | 5 | 0·8521 | 38·9 |

*Experiment XVIII.*—The 10 grams of albumin coagulum used in the digestions contained 1·4333 grams of dry albumin. The mixtures were warmed at 40° C. for the different periods stated, after which the undissolved albumin was filtered off, washed and weighed.

| | Neutralized pineapple juice. | Time. | Undissolved albumin. | Per cent. digested. |
|---|---|---|---|---|
| A | 100 c. c. | ¼ hour | 1·3201 grams | 7·9 |
| B | 100 | ½ | 1·2472 | 13·0 |
| C | 100 | 1 | 1·1554 | 19·4 |
| D | 100 | 2 | 1·1366 | 20·7 |
| E | 100 | 4 | 1·0117 | 29·5 |
| F | 100 | 6 | 1·0012 | 30·2 |

While the results of these two experiments differ somewhat from each other in some respects, they are alike in showing that the ferment commences to act upon the proteid matter at once, and that this digestive action steadily continues, in the case of the above proteid, for about four hours, after which time the action becomes very much slower.

*Separation of the proteolytic ferment.*

Saturation of neutralized pineapple juice with ammonium sulphate precipitates, as already stated, all of the proteid matter contained in the solution. The filtrate does not give the slightest trace of a turbidity on boiling, even after the addition of acetic acid. Nitric acid likewise fails to give any reaction. Acetic acid and potassium ferrocyanide, however, give a slight flocky precipitate, which does not appear to be composed of proteid matter. The ammonium sulphate precipitate contains all of the proteolytic ferment and likewise the milk-curdling ferment, if this is a distinct body. It is readily and completely soluble in water, and by long continued dialysis the solution can be freed from ammonium sulphate. Unless every trace of adherent salt is removed from the solution, the fluid remains fairly clear, but the reaction so far as our experience extends becomes, almost invariably, slightly alkaline. On evaporation of the fluid, after removal of all, or nearly all of the salt, a scaly residue is obtained readily soluble in water, or in the slight trace of salt present, with strong proteolytic power and giving distinct reactions with the xanthoprotein, biuret and Millon's test. The aqueous solution is usually slightly turbid, the turbidity however disappearing on the addition of a little salt-solution, especially with the aid of a gentle heat, also on the addition of 0·2 per cent. hydrochloric acid. Evidently, the bodies composing the ammonium sulphate precipitate are readily soluble in very dilute salt-solution, if not in water alone.

This method constitutes a fairly good way of separating the ferments from the bulk of the extraneous matters present in the juice. We have not, however, spent much time in a close study of the make-up of this product as it is obviously a mixture of essentially all the proteid bodies contained in the juice, but it makes a very good preparation with which to demonstrate the proteolytic and milk-curdling properties of the ferments.

The most satisfactory method we have thus far found for the isolation of the proteolytic ferment, and one which yields a product with very strong digestive power, is by precipitation with common salt.

Saturation of neutralized pineapple juice with sodium chloride gives rise to a small flocculent precipitate, which is not at all increased by the addition of acetic acid to the salt-saturated fluid. Obviously, this precipitate might be composed of a globulin, or of a body akin to heteroproteose. It was studied after the following

plan : a large volume of freshly filtered juice was carefully neutralized with sodium carbonate, and then saturated at the temperature of the room with pure salt. The slight flocculent precipitate which resulted was filtered off, washed with saturated salt-solution, then dissolved in water and dialyzed in running water, putrefaction being prevented by addition of thymol. After several days' dialysis, though still containing some sodium chloride, a portion was tested as follows : it had the appearance of a somewhat turbid fluid, as though a separation had commenced to take place, but it could not be filtered clear. On warming the mixture gently, the turbidity showed a tendency to clear up somewhat. Subjected to careful heating, the mixture appeared to show an increased turbidity at 65° C., while at 72° C. there was a distinct and heavy turbity, but no separation of flocks. As the temperature was raised, the turbidity changed to a thick milkiness without, however, any appearance of flocks even on boiling. The solution seemed perfectly neutral to delicate test papers. By judicious addition of dilute acetic acid and renewed heating, the milky fluid was finally made to yield a flocky precipitate, in the filtrate from which no proteid reaction could be obtained, thus indicating that the sodium chloride precipitate is composed wholly of matter precipitable by heat.

The solution taken from the dialyzer showed strong proteolytic action on blood fibrin.

The remainder of the solution was dialyzed for one week longer, until nearly every trace of sodium chloride was removed, and again tested. The fluid had a very milky appearance, but no distinct separation of flocculent matter was seen. This heavy turbidity cleared up somewhat on warming and disappeared completely on adding a few drops of 20 per cent. salt-solution, also on the addition of a drop or two of dilute nitric acid. The addition of more nitric acid to the latter solution was followed by the reappearance of a turbidity, which did not disappear by gentle heat, but separated into flocks, not readily soluble in a large excess of nitric acid.

The reaction of the solution taken from the dialyzer was faintly alkaline. Heated, the solution became distinctly turbid at 65°–70° C. and quite opaque at 80° C., but without separation of flocks until after persistent boiling. A drop of acetic acid, however, added to the boiling fluid quickly caused a flocculent precipitate, the filtrate from which was practically free from all trace of proteids. The salt-free solution, although faintly alkaline, still showed strong proteolytic power.

On cautiously adding 0·2 per cent. hydrochloric acid to the faintly alkaline fluid a heavy turbidity made its appearance, which changed to a flocculent precipitate as the fluid became slightly acid; on addition of a small excess of acid the precipitate quickly dissolved. From this it may be inferred that if the solution had been perfectly neutral, a more pronounced separation of the proteid might have occurred in the dialyzer tube; evidently, however, the body is extremely soluble in very dilute acid and alkaline solutions, as well as in dilute solutions of neutral salts. Thus, the precipitate produced in this manner by the addition of a little 0·2 per cent. hydrochloric acid was readily dissolved by 10 per cent. salt-solution, the fluid becoming turbid again on boiling, with separation of a flocculent precipitate.

Further, by gently warming the turbid mixture resulting from the addition of a few drops of 0·2 per cent. hydrochloric acid, the fluid cleared up almostly completely, the turbidity returning as the mixture cooled.

Addition of dilute hydrochloric acid, however, did not precipitate all of the proteid; the filtrate still contained some proteid matter and, moreover, showed marked proteolytic action on fibrin. Thus in one instance, 0·2 per cent. hydrochloric acid was added to the dialyzed solution until a distinct flocky precipitate resulted, this was filtered off and dissolved in 10-per cent. solution of sodium chloride. This solution on being heated gradually, grew turbid at 66°–68° C. but did not yield any further precipitate even on boiling. The turbidity did not disappear on the addition of 0·5 per cent. sodium carbonate, or of 0·2 per cent. hydrochloric acid, but was soluble in dilute potassium hydroxide.

The filtrate from the above precipitate showed marked proteolytic action; it also yielded a slight precipitate with strong nitric acid, not dissolved by warming; subjected to heat precipitation the solution grew turbid at 73° C., but did not give any further precipitate even on boiling. The turbidity did not readily disappear on the addition of either dilute hydrochloric acid or of 0·5 per cent. sodium carbonate, but was quickly soluble in very dilute potassium hydroxide. Evidently, the substance precipitated by the acid and that remaining in solution were essentially the same.

From the foregoing, it is plain that the proteid substance precipitated by saturation of neutral pineapple juice with sodium chloride, is a peculiar body partaking both of the characters of a globulin and of heteroproteose. That it approximates more closely to the latter

is evident from the fact that the several precipitates produced by heat are more soluble in dilute alkalies than is customary for coagulated globulin, and that the precipitate produced by addition of dilute hydrochloric acid is so readily soluble on warming the neutral or slightly acid mixture, followed by its re-appearance as the temperature is lowered. On the other hand, the pronounced insolubility of the proteid in warm nitric acid of all strengths is contrary to the usual behavior of the proteoses. Further, the substance gives a biuret reaction more violet than red, and when boiled for a short time with 0·5 per cent. sodium carbonate it is transformed apparently into alkali-albumin, since the precipitate resulting from neutralization of the alkaline fluid is then insoluble in salt solution, although readily dissolved by a slight excess of dilute hydrochloric acid.

It is further evident that the proteolytic ferment of pineapple juice is either associated with this peculiar globulin or proteose-like body, or else is the body itself. The above method of precipitating the proteolytic ferment, by saturation of the juice with sodium chloride, brings about a complete separation of the ferment, provided the fluid is fully saturated with the salt. The filtrate, however, still contains some proteid matter, precipitable by shaking the fluid with neutral ammonium sulphate. This substance was separated from a large quantity of juice, by treating the filtrate from the sodium chloride precipitate with ammonium sulphate, added to complete saturation.

The slight stringy precipitate which resulted was filtered off, dissolved in water and dialyzed. After several days a small portion of the solution, though still containing some salts, was tested as follows: heated gradually the solution became slightly turbid at 65°–70° C., with separation of flocks at 80°–82° C. The filtrate from this precipitate gave no further signs of separation even when heated to boiling, but a drop of acetic acid added to the hot fluid produced a slight turbidity.

The dialysis was continued for about ten days longer, until the salts were almost wholly removed, when the solution was found faintly alkaline and slightly turbid. This turbidity disappeared at once on addition of a drop of dilute nitric acid, also on the addition of a few drops of salt solution. Addition of 0·2 per cent. hydrochloric acid to faint acid reaction produced a slight precipitate, readily soluble in salt solution. Subjected to heat precipitation, the turbid fluid cleared up somewhat at first, then became slightly turbid

at about 68° C., the turbidity becoming more pronounced at 75°–80° C., followed by the separation of a fine flocculent precipitate at 85°–87° C. A more complete separation was obtained by adding a drop of acetic acid to the hot fluid. The precipitate produced by heat at about 85° C. was almost wholly insoluble in dilute alkalies.

This ammonium sulphate precipitate was found wholly free from any proteolytic power either in neutral, acid or alkaline solutions. With the biuret test it gave a very faint violet color.

Hence, as already stated, saturation of neutral pineapple juice with sodium chloride precipitates all of the proteolytic ferment present, while there remains in solution some proteid substance, precipitable by ammonium sulphate, having essentially the same chemical properties as the preceding body. Certain points of difference, however, are noticeable; thus the body precipitated by sodium chloride shows more of a tendency to separate from its solution on dialysis, than the substance precipitated by ammonium sulphate. Further, the former body on exposure to heat precipitation separates less readily from its solution, except on the addition of a drop of acid, although the solution shows a heavy turbidity at much the same temperature as the latter body. The one thing, however, to be emphasized here is that the sodium chloride precipitate is strongly proteolytic, while the body separated by ammonium sulphate is devoid of this property. A more exact study of the chemical status of these two substances is now in progress, but at present nothing more definite can be said.

Another method of separating the proteolytic ferment is by precipitation with magnesium sulphate. Saturation of neutralized pineapple juice with this salt gives much the same separation as that produced by saturation with sodium chloride; i. e. a slight flocky precipitate, readily soluble in water and, after removal of the adherent salt, strongly proteolytic. The filtrate still contains some proteid matter, precipitable in small quantity by the addition of sodium sulphate in substance.

The magnesium sulphate precipitate was examined with the following results; it was dissolved in water and dialyzed for several days, until the greater portion of the adherent salt was removed. On testing the solution by heat precipitation, it grew distinctly turbid at 62° C., with separation of flocks at 75° C. This precipitate represented practically all of the proteid present in the solution, for boiling the filtrate, with or without acid, failed to give any further separation other than a faint turbidity produced by the

acetic acid. The dialysis was continued for a week or more longer, until every trace of sulphate was removed, when the reaction of the solution was found distinctly alkaline, so much so that no precipitate separated even on boiling the solution, although it became quite opaque at about 78° C., until a drop of acetic acid was added. In another preparation, where the alkalinity was less pronounced, the solution after dialysis of all, or nearly all of the salt, gave a flocculent precipitate at 85° C.

The faintly alkaline solution of the proteid yielded a precipitate on addition of 0·2 per cent. hydrochloric acid, very soluble in a little salt solution and in a slight excess of 0·2 per cent. acid. Only a portion of the substance was separated however, by this method of precipitation. The filtrate, which appeared nearly neutral to test papers, on being heated grew turbid at 73° C., without however any separation of flocks until the solution was boiled. The slight flocculent precipitate which then resulted was wholly soluble in 0·5 per cent. sodium carbonate, and nearly so in 0·2 per cent. hydrochloric acid. Further, the filtrate had marked proteolytic power and treated with nitric acid gave a distinct turbidity, not appreciably diminished by warming the mixture. That portion of the proteid precipitated by the dilute hydrochloric acid gave, after solution in dilute sodium chloride, essentially the same reactions as the foregoing, so that evidently the two fractions were identical.

This preparation of the ferment agreed fairly well in its reactions with the substance separated by sodium chloride. Like the latter, the turbid solution resulting from the dialysis cleared up on addition of a little salt solution, likewise on the addition of a few drops of dilute nitric acid, while more acid produced a second turbidity not readily dissolved by an excess of the acid, or by heat. With the biuret test, a more distinctly reddish color was produced than heretofore seen.

There are, therefore, two good methods for the isolation of the proteolytic ferment from pineapple juice; one by saturation of the neutralized fluid with sodium chloride, the other by saturation with magnesium sulphate. In our opinion, the former method yields a product with the strongest proteolytic power, although this needs to be verified by further observations. As to the exact chemical nature of the ferment, or of the body it is associated with, we are not yet able to speak with perfect confidence. It is our opinion that the proteolytic body separated by the above methods is a mixture of a globulin and a proteose, but as yet we have not been able to accomplish a dis-

tinct separation. On the other hand, it is possible that the substance is a single body possessed of properties akin to both the above, but this view seems hardly probable; its precipitation by saturation with magnesium sulphate and by sodium chloride possibly favor its being a globulin, while its extreme solubility in dilute salt solutions, in dilute acids and alkali are equally characteristic of both bodies. The more or less constant solubility of the heat precipitates in dilute alkalies favors its proteose nature, and if a proteose it is most closely related to heteroproteose. The substances are, moreover, alkaline reacting bodies completely precipitable by heat, especially in the presence of a trace of acid.

We are now occupied, with the aid of larger quantities of material, in an attempt at a better separation of these bodies with the hope of acquiring more definite knowledge regarding their chemical nature, composition, etc.

Whether globulin or proteose, these bodies present in pineapple juice, are very resistant to the digestive action of the ferment. Thus, long continued warming (3–5 hours) of fresh pineapple juice with strong proteolytic power at 40° C. does not, in the least, change the temperature at which the several heat precipitations occur; a point which certainly indicates the resistance of these proteids to proteolytic, or at least to this particular kind of proteolytic, action; the bodies in this respect resembling the *atmid* bodies described by Neumeister.*

*Products formed by the proteolytic action of the ferment.*

On this point, we have made only a few preliminary experiments, designed simply to throw some light on the nature of the ferment as a proteolytic agent. The results indicate that the ferment is more nearly related to trypsin than to pepsin, in that not only are proteoses and peptone formed by its action, but also leucin and tyrosin.

When washed and boiled blood fibrin, for example, is warmed at 40° C. with fresh pineapple juice of *natural acidity* for two or three hours, the proteid matter is thoroughly digested, but, as previously stated, a fairly large residue of finely divided matter remains undissolved, resistant to the further action of the ferment. This antialbumid-like matter is readily soluble in weak solutions of sodium carbonate, from which it is re-precipitated by addition of acetic acid,

* Zeitschrift für Biologie, Band xxvi, p. 57. "Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Erweisskörper und Albumosen."

and not readily dissolved by an excess of the acid. This same body is likewise formed by the action of fresh pineapple juice in neutral or weak alkaline solutions, and also by the isolated ferment as prepared by the methods previously described.

Boiling the acid digestive fluid gives no noticeable coagulum, and addition of moderately strong nitric acid likewise fails to produce any precipitate. Neutralization of the acid digestive mixture may give rise to a small amount of a neutralization precipitate, resembling acid-albumin. On evaporation of the neutralized fluid, the solution remains fairly clear, and when sufficiently concentrated addition of strong alcohol gives a gummy precipitate of proteose and peptone, while from the alcoholic solution crystals of tyrosin can be obtained and, in lesser quantity, leucin also. Tyrosin appears to be present in much larger amount than leucin. On dissolving the alcohol precipitate in water and saturating the solution with ammonium sulphate a heavy precipitate of proteose is obtained, while in the filtrate a large amount of true peptone is found, which after removal of the sulphate by dialysis gives a bright red color with the biuret test, etc. So far as our experiments extend, the proteose is composed mainly of a deutero-like body, only a small precipitate being obtained by saturation with sodium chloride, either in a neutral or slightly acid solution. An experiment like the preceding carried out with coagulated egg-albumin gave essentially the same results.

When pineapple juice of *neutral or faintly alkaline reaction*, or a *neutral or slightly alkaline solution* of the proteolytic ferment, is warmed with blood fibrin, for example, the digestion appears much the same as the preceding, but on heating the filtered solution a very pronounced milky turbidity appears, which on addition of a drop or two of acetic acid changes to a heavy flocculent precipitate, insoluble in excess of the acid.

Further, addition of nitric acid to the neutral or slightly alkaline digestive fluid gives a heavy curdy white precipitate, insoluble on application of heat, and likewise insoluble in an excess of the acid. Heated with an excess of acid, the precipitate takes on an intense yellow color. This precipitate produced by nitric acid is likewise insoluble in a 10-per cent. solution of sodium chloride, at least in the presence of the acid.

This body, so characteristic of bromelin digestion in *neutral or faintly alkaline* solutions, is very resistant to the action of the ferment, being still present even after a long-continued digestion. It seems probable that this substance results from the action of the fer-

ment in a neutral or alkaline solution, on the insoluble antialbumid-like body so conspicuous in an acid digestion; for while the latter body is also present in neutral and alkaline digestions, the amount seems smaller, and further, the reactions of the two bodies are very much alike.

Aside from this one point of difference, the products formed in a neutral or alkaline digestion are, so far as we have seen, essentially the same as those formed by pineapple juice of natural acidity. Thus, on boiling a slightly alkaline digestive mixture resulting from the action of the isolated ferment on blood fibrin, and adding a drop or two of acetic acid to facilitate the separation of the coagulum, a clear filtrate was obtained, without trace of reaction with nitric acid, except in the presence of salt, and giving the usual reactions for proteose and peptone. Thus, the addition of concentrated salt-solution to the clear filtrate, followed by a few drops of acid gave more or less of a turbidity, readily dissolved on application of heat, reappearing on cooling.

We hope soon to present a more detailed report regarding the properties and composition of the several products resulting from the action of the isolated ferment on egg-albumin, blood fibrin and myosin.

Sheffield Biological Laboratory of Yale University.

## XVIII.—The Nephrostomes of Rana. By Oliver C. Farrington.

In the Urodela, as is well known, nephrostomes are always present, serving as a means of free communication between the body cavity and uriniferous tubules. According to Hoffman,* both Spengel and Meyer, whose elaborate investigations independently made, first gave us accurate knowledge of the structure and office of the nephrostomes of Urodela, state that these organs exist also in the Anura, but they were unable to determine with certainty the point of connection between them and the tubules. The nephrostomes appear, according to these authors, on the ventral surface of the kidney, where this is covered by the peritoneum. Their number varies in different genera, but in Rana there are from 200 to 250. In most genera they open directly inward, but in some they take a short horizontal course on the surface before entering the kidney. As before stated, their point of junction with the tubule is difficult to establish with certainty, but according to the view of Spengel they unite with the fourth section of the tubule rather than with the first section or neck as in Urodela. Nussbaum also investigated this question and his first results† confirmed Spengel's views. Later investigations,‡ however, led him to the belief that although during the tadpole stage the nephrostomes connect with the neck of the tubule, as development proceeds they are forced away from it and open, in the adult frog, into the branches of the renal-portal vein. Haslam,§ the most recent writer on the subject, controverts all the previous views, stating that neither in microscopic sections or teased preparations has he been able to find any trace of such organs. The conclusions which he draws from various experiments on the frog are as follows: "If the peritoneal funnels exist in the adult frog, (1) they are very difficult to find; (2) they do not form a free communicating path between any part of the uriniferous tubules and the abdominal cavity; (3) their superficial terminations have no free cilia." Heidenhain, according to this author, was also unable to find these organs.

* Bronn's Klassen und Ordnungen des Thier-reichs, vol. vi, p. 461.

† Sitzungsb. d. Niederrheinischen Gesell. in Bonn, 1877, p. 122.

‡ Zool. Anzeiger, 1880, No. 67, p. 514.

§ Ecker's Anatomy of the Frog. Translated by Geo. Haslam, 1889, p. 336.

The subject is one of interest, since it is desirable that we should know at what point in the ascending scale of animal life these organs, which probably exist in all vertebrates as an embryonic feature, cease to characterize the adult forms.

In the hope that the recent improved methods of section cutting and reconstruction might throw additional light on the question, the work which is described in the following pages has been done by the writer.

In carrying on this work, all the experiments which were made on the frog were duplicated by similar ones on the newt, and the same is true of all the preparations made for section cutting. This was done in order to compare directly results obtained in a species where the nephrostomes are known to exist, with those given by the frog.

Two species of Rana were used, *Rana Catesbiana* and *Rana virescens*, but no dissimilar results due to difference of species were noted. Of the newt, the species used was the common *Diemyctylus virescens.*

The investigation has led to the following conclusions:

I. Microscopic sections of the frog's kidney often show, passing inward from the ventral surface, small tubes which are to be regarded as nephrostomes.

II. These tubes have free, active cilia.

III. Strong, if not conclusive evidence exists to show that these open into the blood capillaries of the kidney.

I. Fig. 1 shows a section of the newt's kidney with nephrostomes appearing at various points indicated by the letter *n*.

At the surface these have a diameter of about $\cdot 04^{mm}$, then passing inward show a slight funnel-like enlargement to about $\cdot 06^{mm}$. For this part of its course the tube is seen to be made up of a ciliated epithelium, the cells of which are narrow, elongated, somewhat fusiform, and have indistinct nuclei. Two of these funnels are seen uniting in a common tube, in fig. 1, an arrangement stated by Spengel to be quite common. For the rest of its course the tube, having a diameter of about $\cdot 024^{mm}$, loses its distinctive epithelium and its walls are thin and structureless till it opens into the uriniferous tubule (*n. t.* fig. 1). Figs. 2, 3 and 4 show sections of the kidney of the frog prepared similarly to those of the newt. As will be seen, these show openings or cavities on the ventral surface, about $\cdot 035^{mm}$ in diameter, lined with a distinctive epithelium and resembling in every respect those just noted in the newt. The distance to which

they can be traced inward depends on the favorableness of the section. The one shown in fig. 3 extends inward for a distance of ·16$^{mm}$. By tracing the openings onward through successive sections we find them passing into a tube of an average diameter of ·06$^{mm}$, which continues its course near the ventral surface of the kidney (*n. t.* fig. 2). The tube finally loses its distinctive structure and opens probably into the capillaries flowing toward the vena cava inferior. This inner termination, however, it was found impossible to trace with certainty, though it was sought for with the greatest care through a large number of sections. The opening at the point of junction is undoubtedly extremely small, so that the chances of finding a section in which it could be traced with certainty would be few indeed. The nature of the epithelium lining the nephrostome is shown in fig. 3. It is seen to be made up of large cuboidal cells having well defined nuclei and resembling in size and shape the cells of the uriniferous tubules, though they lack the striations which usually characterize these. At the opening on the surface the cells become flattened and smaller and the cell outline is less distinct.

The nephrostomes of tadpoles were found to possess a similar epithelium, but in the tadpoles the cells are more nearly like those of the uriniferous tubules than in the adult frog.

The method of preparation of these kidneys and of those of the frog was to preserve in Müller's fluid 24 hrs., transfer to 95 per cent. alcohol and stain with hæmatoxylin.

II. The cilia appear so plainly in sections that there can be no doubt of their presence. Further evidence of ciliary action was obtained, however, by adoption of the method proposed by Nussbaum (l. c.), which he describes as follows: "Chloroform the animal until respiration has ceased. Inject in the body cavity 0·5 per cent. salt solution containing powdered carmine. Close the body wall. Immerse the animal for 3 hrs. in Muller's fluid and after 12–24 hrs. transfer the kidney to alcohol."

As Nussbaum states, thin sections of the kidney will then show the carmine distributed through the funnels and tubes, thus giving positive evidence that its fine particles have been carried inward by ciliary action. Such a section is shown in fig. 2. Haslam states (l. c.) that having placed properly dissected frogs in 0·6 per cent. sodium chloride solution in which finely divided gamboge was suspended, no trace of ciliary action was in any case found on either surface of the kidney. In what way it was anticipated that the ciliary action would show itself is not stated, but it is doubtful if the cilia of

openings so minute could produce currents or accumulations of particles large enough to be seen from the surface. Applying the experiment of Nussbaum, however, at seven different times to both male and female frogs of various sizes, the evidences of ciliary action previously described were observed in every case.

III. Our belief that the nephrostomes in the frog open into the blood capillaries of the kidney, rests mainly on the fact that sections prepared after Nussbaum's method show that the carmine is present not only in the nephrostomes, but in the blood capillaries and large veins which are near the ventral surface of the kidney. This is shown in both figs. 2 and 4. Applying the method of Nussbaum to the newt, we find the carmine invariably in the uriniferous tubules and not in any other part of the kidney. In the frog, however, the carmine is not found in the tubules at all but only in the blood vessels. This makes the evidence almost conclusive that the nephrostomes open into the tubules in the one case and into the blood vessels in the other. We need, however, to trace the tube directly to its termination before we can assert positively its connection, since it may be possible that the carmine could make its way into the circulation by some other course. Nussbaum states that by sections cut in glycerine he was able to follow directly the course of the carmine from the nephrostome into the blood vessel. It is very doubtful, however, if the evidence from such sections could be relied upon, since the writer found that unless some fixative for the carmine was used the particles were scattered by the knife through every part of the kidney. To overcome this difficulty use was made of a method for imbedding proposed by Prof. S. I. Smith. The kidney was imbedded in celloidin, but instead of trying to make sections of this block by the usual method of cutting under alcohol, the block was reimbedded in paraffine. From this block, then, sections could be cut as usual with paraffine, and the celloidin held all particles so firmly in place that there could be no doubt that the place of the carmine in any part was that it had naturally reached. From fig. 4, which represents a section prepared after this method, it will be seen that the carmine appears only in those blood vessels near the ventral surface. This was invariably true, and hence the conclusion that the nephrostomes open into the capillaries flowing into the vena cava. This fact, moreover, makes it quite unlikely that the carmine could have entered the circulation in any other way than through the nephrostomes, for it would then be equally distributed through the blood vessels of the kidney. To test this point further, the vena cava inferior of a live frog was tied

just above the kidney before injection of the carmine. It was hoped thus to determine whether the carmine would enter the circulation through other vessels, if it were not supplied by the kidneys. Upon examination the particles were found to have entered the nephrostomes only a little way, and were not present in any of the blood vessels; but, as the stopping of the circulation caused the animal to die very quickly, the test was not regarded decisive.

It seems probable that if the nephrostomes connect with the blood vessels they would be reached by injections of the latter. As no mention is made by other writers of experiments of this kind, except that of the injection of the ureters by Haslam, this point was carefully tested. The first injection was made through the vena cava inferior, the anterior abdominal being also opened. This showed only the blood vessels to be injected and no passage of the fluid into any of the nephrostome tubes. The second injection was made in the same way except that the renal-portal of one side was tied so that if any extra pressure were needed to force the fluid into the tubes it might be thus supplied. The results were, however, like those of the first injection. The third injection was made through the renal-portal of a very large frog with the anterior abdominal opened. By this means the nephrostomes were successfully injected, the fluid showing plainly in the tubes and funnels and even coming to the surface, though it did not flow out to any extent. This result seems to place the connection of the nephrostomes with the blood vessels almost beyond question. On seeking, however, to trace the direct opening of the nephrostome tube into a blood vessel by means of sections, it was found as before impossible to do so with absolute certainty, though the connection was in many cases very close. Since the nephrostomes probably serve as lymph vessels, the openings into the capillaries must be small enough to prevent the passage of the blood corpuscles into them, so that, as before stated, the chances of tracing them with certainty are few indeed. The experiments seem to teach that the passages inward from the nephrostomes can be reached by a current going toward the vena cava but are closed to one coming from it. This is difficult to explain if true, but needs further proof. The remarkable change of function which the nephrostomes seem to undergo, from service as excretory organs to that of lymph vessels, gives rise, also, to physiological questions of much interest. It is not, however, the purpose of this paper to discuss these, since they call for much further investigation. It is the hope of the author that others may study these points and bring new facts to light.

A few points may be mentioned regarding the macroscopic appearance of the nephrostomes. The writer found they could be seen plainly from the surface by staining the kidney in 0·5 per cent. silver nitrate solution from 3 to 5 minutes. By this treatment the nephrostomes are made to appear like minute *craters* scattered over the surface. As noted by Hoffman, they are most abundant in the region medianward from the adrenal gland and are especially numerous near the large branches of the vena cava. Fig. 5 is an attempt to represent their distribution and appearance, though it is difficult to do this by a drawing. The nephrostomes are represented by dots and the little circles indicate lighter spots on the surface which are often mistaken for their openings but are in reality produced by the Malpighian capsules showing through. The largest number of nephrostome openings counted on any one kidney was 150.

In conclusion the writer wishes to acknowledge his indebtedness to Prof. S. I. Smith, at whose suggestion the investigation was undertaken and whose valuable advice and assistance have been freely rendered during the work.

Sheffield Biological Laboratory, June, 1891.

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## EXPLANATION OF PLATE XXIV.

Fig. 1. Dorso-ventral section of kidney of *Diemyctylus virescens*. 9$\mu$ in thickness.

Fig. 2. Ventral portion of dorso-ventral section of kidney of *Rana Catesbiana*. 9$\mu$ in thickness.

Fig. 3. Similar section showing single nephrostome. 6$\mu$ in thickness.

Fig. 4. Similar section entire, showing the distribution of the carmine in the blood vessels.

Fig. 5. Right kidney of *Rana Catesbiana*, female. Ventral view.

*a.* artery; *b. v.* blood vessel; *c.* carmine; *M.* Malpighian capsule; *n.* nephrostome; *n. t.* nephrostome tube; *u. t.* uriniferous tubule; *v. i.* large vein leading to vena cava. The ventral surface in the figures is to the left, dorsal to the right. The carmine where not lettered is represented by black dots.

## XIX.—Notes on the Fauna of the Island of Dominica, British West Indies, with Lists of the Species obtained and observed by G. E. and A. H. Verrill. By G. E. Verrill.

The following collections were obtained by my brother and myself during March, April, and May, 1890, on the Island of Dominica, one of the Lesser Antilles, situated in Lat. 15° 18′ to 15° 45′ N. and Long. 61° 14′ to 61° 30′ W.

This island lies approximately north and south, is of volcanic origin, rising abruptly from the sea, and is about 29 miles long by 17 wide, the whole containing about 290 square miles. Generally speaking it is composed of a high central ridge or crest running lengthwise, the crest itself being made up of a number of peaks, the highest attaining an altitude of about 5300 feet.

From this central crest it slopes down to the east and west in a succession of lower peaks and ridges with deep valleys and ravines between, many of which contain rapid mountain streams. Close to the coast these valleys widen, frequently to quite an extent, giving chance for sugar plantations, etc. This is particularly the case on the western, or leeward side, where the slope is much less than on the windward, or Atlantic side, so that it is possible to cultivate the ground well up on the mountains on the former side.

The vegetation of the island is of the most luxuriant type, covering the mountains to their tops and consisting of a very great variety of trees, palms, tree-ferns, shrubs, vines, etc. The tree-ferns are very abundant and often twenty feet or more high. Many of the trees reach a great height, while the smaller ones with the various vines and lianas form a network almost impassable unless a person is armed with a "cutlass" or "machete" almost invariably carried by the natives to cut their way.

The temperature along the coast averages about 85° F. most of the time, but back in the interior, among the mountains, it is much cooler; so cool, in fact, that at night one needs a pair of good heavy blankets. The climate is also very damp, especially up in the mountains where, even during the dryest portion of the year, there is a succession of short, sharp showers every day, each followed by

bright sunshine. In fact it not unfrequently rains from a cloudless sky, so that one is no sooner dry from one shower than he is pretty sure to be drenched by another.

This island appears to have been but very little visited by collectors. So far as I am aware but one collection of any size, that of Mr. Fred. A. Ober, made in 1877 for the Smithsonian Institution, has previously been sent to this country (cf. Proc. U. S. Nat. Mus., vol. i, p. 48.). To this Dr. H. A. A. Nicholls subsequently made some additions (ib. vol. iii, p. 254). Another collection was, however, made there in 1887 and 1888 by Mr. Geo. A. Ramage, the naturalist employed by the joint committee of the Royal Society and British Association for investigation of the fauna and flora of the Lesser Antilles; and in 1863 Rev. E. C. Taylor spent two weeks in Dominica making a small collection (cf. Ibis, vol. vi, p. 157).

The island itself presents many difficulties to a collector; for, aside from its being very mountainous and heavily wooded, as mentioned above, there are no means of transportation for baggage, etc., except on the heads of natives; while the collector himself must depend upon his own legs or one of the small, and not too lively, native ponies, there being but two carriages on the island and their use being restricted to the immediate vicinity of the towns and along the coast for a few miles, as there are no carriage roads whatever in the interior, or crossing the island, and in many places even the trails are very poor.

Our collections were, for the most part, made from four camps, as follows: at Laudat, a little hamlet at an elevation of about 1600 feet, at the head of the Roseau Valley, and about ten miles from Roseau, the principal town on the island; at Spring Hill, situated on the side of the same valley at about the same elevation as Laudat, or a little less, and distant about three miles from the latter place; at Bass-en-ville, situated on a high plateau in the interior of the island, about half way across, and at an elevation of about 2000 feet; and at Lasswa, situated on the eastern or windward side of the island.

We found the inhabitants uniformly kind, courteous, and anxious to help us in every way. Our thanks are especially due to the Governor of the Leeward Islands, and to the President and Members of the Council of Dominica for their kindness in giving us permission to collect there, placing at our disposal the house for the Government engineers at Bass-en-ville, and for courtesy in many ways. We are no less indebted to the following named gentlemen residing on the island:—to Mr. James, the Inspector of Police, for valuable aid in many ways; to Dr. H. A. A. Nicholls, well known by his contribu-

tions to science from the fauna and flora of this island, for aiding us by his own knowledge of the birds, the best collecting grounds, and much general information and help; to the Bishop of the Roman Catholic Church and his priests for their hospitality and aid, especially in allowing us to enter the churches in search of some species of bats and the owl (*A. flammea nigrescens* Lawr.); to Mr. A. Davis Reviere and his brother Emory Reviere, of Clark Hall, for their great kindness and hospitality while we were in the Layou Valley; to Mr. Hennessey Dupigny, who accompanied us part of the time, and to whose consummate woodcraft and knowledge of the habits of the birds we are in a large measure indebted for our success and for many valuable specimens, which we should otherwise have been unable to procure; and to Mr. Arthur Ogilvy, Mr. A. Frampton, and Mr. Wm. Gellion, for many acts of kindness and aid. In addition to these above named gentlemen many others gave us much valuable assistance and all endeavored to make our stay there as pleasant and profitable as possible.

For the working up and identification of the collections we are indebted to the following gentlemen:—to Mr. J. A. Allen of the Am. Mus. of Nat. Hist. for the identification of the birds and mammals, for certain notes on the same which appear in the following list, and for valuable advice on the nomenclature used therein; to Prof. E. D. Cope for the identification of the Reptiles and Batrachians, and for notes on the same; to Mr. Sanderson Smith of the Am. Mus. of Nat. Hist. for the identification of part of the Mollusca; and to Prof. S. I. Smith of Yale Univ. for the identification of the Crustacea.

## *Notes on the Mammals.*

The mammals of Dominica are very few indeed, those indigenous being mainly bats, of which there are several species that are quite common, but rather difficult to procure owing to their nocturnal habits. We procured but one, which Mr. Allen examined and says is probably *Vespertilio nigricans*, at least it so nearly resembles it that he cannot see wherein it differs without having more material.

These small bats, of this species or very closely resembling it, were very common about Bass-en-ville in the evening, but though I shot several I lost all but this one among the high grass and bushes, the light being very dim when they appeared, and in the morning we could never find any trace of them, they probably having been eaten or carried off by the crabs (*P. dentata*) which were always on

the watch for anything in the nature of meat that we might throw out. Besides these small bats we several times observed much larger ones of one or more species which I suppose were some kind of fruit bats, but we were unable to procure any, as we never saw them except when it was quite dark, too dark to shoot with any accuracy at an object moving so swiftly and seen for so short a time as they were when suddenly darting across the narrow trail bordered by thick woods on each side. These large bats were seen almost exclusively at a short distance from the coast, and I think they are confined to the less elevated portions of the island.

Besides the bats, there are found here a species of agouti and an opossum, or opossum-like animal, called "Manacou" by the natives, the latter having been introduced, but both are now quite common, and the agouti forms quite an article of diet among the natives. The common rats and mice are exceedingly abundant, but we saw no native ones at all.

## *List of Birds obtained and observed, with Notes on their Habits, Nests, and Eggs.*

PLATES XXV, XXVI, XXVII.

The following notes on the habits are from the combined observations of my brother, A. H. Verrill, and myself. The descriptions of the nests and eggs themselves, unless otherwise stated, I have made from the specimens actually obtained by my brother and now in the Peabody Museum, but the notes on the nesting habits, etc., are mainly by him.*

Unless otherwise stated the measurements given were made from the fresh specimens at the time of collection, and are given in inches in the following order: length, wing, tail, extent. In some cases, where they may be useful for comparison, I have added measurements of the bill (exposed culmen unless otherwise stated), and tarsus, both made from the skins. The measurements of the eggs are in decimals of an inch. In most cases I have given the measurements of several specimens to show the variation.

The sex was carefully determined in all cases by dissection, and in one or two cases the previous conclusions with regard to the plumage of the adult female are shown to have been erroneous.

* As I did not arrive in Dominica until the latter part of April, when the breeding season was nearly over, we took few nests after my arrival.

The identification, as previously stated, was by Mr. J. A. Allen, whose authority is too well known to admit of any doubt, even had he not been aided by many of Mr. Ober's type-specimens.

The list probably comprises very nearly all of the land birds, but comparatively few of the aquatic species, as our time was too limited to warrant spending it on these latter, which are, for the most part, much more widely distributed and better known.

It is a rather striking fact that nearly all of these islands have a number of species of Passerine Birds peculiar to each, though represented in the adjacent ones by closely allied species. The entire absence of woodpeckers on this island, covered with trees, is another peculiarity. In a few cases we have added species not previously known in Dominica to the fauna of this island, and in several cases have taken species seen, but not identified, by Mr. Ober.

The vernacular names, given in this and the following lists, are those used by the natives and are commonly in the French patois of the island. When the native name was evidently derived from the French, yet differs materially from it, I have given the patois pronunciation, expressed as well as possible by English spelling, with the original French word and English equivalent in parentheses. In many cases it has been impossible to even guess at the original from which the native word has come, and in other cases it has undoubtedly been derived from the Caribs, a number of whom still inhabit the island ; but, as both the Patois and Carib languages are wholly spoken ones, I have only been able to preserve the sound, as nearly as possible, by phonetic spelling. The following abbreviations are used : Fr. = French, Pat. = Patois, Ca. = Carib, Eng. = English.

It is a rather peculiar fact that the natives know nearly all the birds and distinguish even closely allied species by different names. They also know and readily recognize them by their notes and are generally able to identify the nests and eggs.

The arrangement used is that adopted by the Amer. Ornith. Union.

## Order, LONGIPENNES.

### Family, LARIDÆ.

1. **Sterna fuliginosa** Gmel. "Twar-oo" (Pat.).

A number of terns, probably of this species, were seen at different times flying about the harbor at Roseau. Several other species of terns were also seen but not near enough to identify with any certainty. No specimens of these, and many other marine birds were obtained, owing to lack of time, as has been previously stated.

## Order, TUBINARES.

### Family, PROCELLARIIDÆ.

Much has been said and many stories are told of the so-called "Diablotin," which the inhabitants of this island say used to make its burrows and nests on the mountain which is called Mt. Diablotin, the highest peak in Dominica. All agree, however, that none have been seen there for a long time, twenty years or more, so that it is very difficult to get a good description of what this bird was like. However, the Bishop of the Roman Catholic Church kindly showed my brother an old book in his possession which contained a picture that showed the "Diablotin" to have been some sort of a petrel, but to what genus or species it belonged it was impossible to tell. Further than this we could learn nothing of importance concerning it. Very likely it was the same as the "Diablotin" of Guadeloupe, (cf. Auk. viii, p. 61).

## Order, STEGANOPODES.

### Family, PHAËTHONTIDÆ.

2. **Phaëthon flavirostris** Brandt.

Common. Breeds in the cliffs along the coast on the leeward side of the island. Though no specimens were taken there is no doubt of the identity of this bird as they could be closely observed.

Mr. Ober took this species in the same localities where it was observed by us.

### Family, PELECANIDÆ.

3. **Pelecanus fuscus** Linn.

Abundant all through the West Indies. Several seen about the island.

### Family, FREGATIDÆ.

4. **Fregata aquila** (Linn.).

Seen several times flying over, high in the air.

## Order, ANSERES.

### Family, ANATIDÆ.

According to the natives, ducks of one or more kinds are sometimes seen, in the fall, in the Mountain Lake and fresh water streams. We ourselves saw none at all.

## Order, HERODIONES.

### Family, ARDEIDÆ.

5. **Ardea herodias** Linn. "Crabier noir," Pat. and Fr. (Black Crab-eater). "Black Crabier," Pat. "Black Gaulin," Pat.

This bird was described to us under the above Patois names by Mr. Hennessy Dupigny and others, and one or two were seen in the river at Bass-en-ville, but no specimens were obtained.

Though not observed by Mr. Ober in Dominica or Guadeloupe, he took it in nearly, if not all, the other islands visited by him, and Dr. L'Herminier records it from both Guadeloupe and Martinique in a "Catalogue des oiseaux observés à la Guadeloupe par le Docteur F. L'Herminier, de 1827 à 1844," given by Mr. Lawrence in connection with Mr. Ober's list (Proc. U. S. Nat. Mus., vol. i, 1878, p. 450).

A smaller "Black Crabier," which was described as being found in Dominica by several of the inhabitants, is probably *A. cærulea*, taken there by Mr. Ober.

6. **Ardea egretta** Gmel. "Aigrette," Pat. and Fr. (Egret). "White Gaulin," Pat.

Not particularly rare along the rivers, especially the Layou River. Seen several times in the neighborhood of Bass-en-ville. Though several were observed no specimens were secured, partly owing to the shyness of the birds themselves and partly to our own lack of time, but I am very sure of the identity, as I am familiar with the above species, having seen and shot it many times in Florida. This bird was not observed by Mr. Ober among the Lesser Antilles at all, though "a large White Heron" was described to him as visiting Barbuda, but Dr. L'Herminier records it from both Guadeloupe and Martinique.

As with the last species, the natives also described another "White Gaulin," smaller than this one, which is probably *A. candidissima*, taken there by Mr. Ober.

7. **Ardea virescens** Linn. "Kialee," Pat. "Green Crabier," Pat.
*Butorides virescens* (Linn.); Taylor, Lawr., and Scl. Lists.

Resident. Common along all the streams. Habits the same as in the U. S.

Irides yellow, legs and feet greenish yellow. ♂ 18–6½–2⅝–25, bill 2⅛, tarsus 2. Sex? (from skin), wing 7¼, tail 2¾, bill 2 3/10, tarsus 2.

8. **Nycticorax violaceus** (Linn.). "Crabier," Pat. and Fr. (Crab-eater). "Night Gaulin," Pat.
*Nyctiardea violacea* (Linn.); Scl. List.

Common, but on account of its nocturnal habits not very often seen. Most of our specimens were procured by Mr. Hennessey Dupigny near Shawford in the Roseau Valley.

Iris orange, legs and feet yellow, bill black. Sexes alike, young very different, somewhat resembling the young of *N. nycticorax nævius.* ♂ 26–12–4½–42, bill 3·1, tarsus 3·6. In other specimens, wing 11½–11⅝, bill 3·0–3·1, tarsus 3·4–3·5.

"Nest built in the tallest trees. Eggs pale bluish green."—(A. H. V.)

## Order, PALUDICOLÆ.

### Family, RALLIDÆ.

9. **Ionornis martinica** (Linn.). "Poule d'eau," Pat. and Fr. (Water-hen).
*Porphyrio martinicus* (Linn.); Lawr. List.

A bird described to us by several of the inhabitants under the above Patois name was very likely this species, a specimen of which was shot there after Mr. Ober's departure but which was saved for him (Proc. U. S. Nat. Mus., vol. i, 1878, p. 197). Said by those who described it to be rare. Given from Guadeloupe and Martinique by Dr. L'Herminier.

## Order, LIMICOLÆ.

### Family, SCOLOPACIDÆ.

10. **Ereunetes pusillus** (Linn.). "Bécasse," Pat. and Fr. (Snipe).
*Ereunetes petrificatus* (Illig.); Lawr. List Birds Lesser Antilles (P. U. S. N. M., vol. i, 1878, p. 488).

A sandpiper seen by us several times, but not procured, was probably this species. Mr. Ober also observed a sandpiper here, "Species undetermined," which Mr. Lawrence referred to this species in his "Catalogue of the Birds noted from the Lesser Antilles by Mr. Ober." Obtained by Dr. Nicholls who states that it is "common at the mouths of the rivers during the hurricane months." (cf. Proc. U. S. Nat. Mus., iii, p. 256).

11. **Actitis macularia** (Linn.).
*Tringoides macularius* (Linn.); Lawr. List.

Rather common. Seen on the rocks in the beds of streams and along their shores. No specimens were taken from lack of time but I have no doubt of the identity of this bird, so familiar to every North American collector. Also taken there by Dr. Nicholls.

## Order, COLUMBÆ.

### Family, COLUMBIDÆ.

12. **Columba corensis** Gmel. "Ramier," Pat. and Fr. (Ringdove). "Black Ramier," Pat.

Common in the heavy woods in the interior and among the mountains. Very arboreal. Mr. Ober states that it "never touches the earth"; whether this be so or not we never observed it except while in the trees or on the wing. Feeds on the fruit and seeds of certain trees, particularly the gommier tree. Being excellent eating it is much hunted for food during the open season, and hence is very shy and hard to shoot. Its loud cooing, resembling that of the domestic pigeon, but much louder, may be heard for a long distance, but even when one has carefully followed it up and stands beneath the very tree where the bird is, it is extremely difficult to actually see it.

Sexes alike. Iris yellow flecked with red, skin around the eyes orange, legs and feet dull coral red, bill red at the base and yellow or horn-color at the tip. ♂ 16½–9–6–24; 16⅜–8½–6¼–26½.

13. **Columba leucocephala** Linn. "White-headed Ramier," Pat.

Rare. When first told of this bird by the native hunters I was inclined to think they were mistaken, as I did not then know of its having ever been recorded from this or any of the adjacent islands, but they were very positive and described it well, but stated that it was very rare though found in the same localities and in company with the common "Ramier." While at Bass-en-ville, however, I, myself, saw several specimens on the wing, the white head showing very plainly. Though in spite of every endeavor we were unable to procure any. I do not think there is any doubt about its identity, and am still in hopes of having one or more sent to me, at no distant date, by our friends in Dominica.

This species is also recorded by Dr. L'Herminier from Guadeloupe and Martinique, the islands adjacent to Dominica, and lying respectively north and south of it. Mr. Ober records it from Antigua and Barbuda, but no further south.

14. **Zenaida martinicana** Bonap. "Tourterelle," Pat. and Fr. (Turtledove).

Abundant, but shy and retiring. Found principally near the coast, apparently not reaching to any great altitude. Like the "Ramier" and "Perdrix" it is much hunted as a game bird and, like the latter, is often kept in captivity.

Sexes alike. Iris brown, bill black, legs and feet red. ♂ $12\frac{1}{4}$–$6\frac{1}{2}$–$4\frac{1}{2}$–20; 13–$6\frac{1}{4}$–$4\frac{1}{8}$. ♀ 13–$6\frac{3}{8}$–$4\frac{5}{8}$.

15. **Columbigallina passerina** (Linn.). "Ortolan," Pat. and Fr. "Ground Dove," Eng.

*Chamæpelia passerina* (Linn.); Lawr. and Scl. Lists.

Abundant along the edges of the cane-fields and in the roads along the coast, particularly on the leeward side of the island. Not found among the mountains. Quite tame and unsuspicious. Many are captured and sold for cage birds.

Sexes similar. Iris orange, legs and feet flesh-color, bill brown, lower mandible lighter at the base. ♂ 7–$3\frac{1}{4}$–$2\frac{1}{2}$; 7–$3\frac{1}{16}$–$2\frac{1}{2}$; 7–$3\frac{1}{8}$–$2\frac{1}{2}$; 7–$3\frac{3}{16}$–$2\frac{1}{2}$. ♀ $7\frac{1}{4}$–$3\frac{1}{4}$–$2\frac{1}{4}$–10; 7–$3\frac{1}{8}$–$2\frac{1}{2}$. In all the specimens, exposed culmen $\frac{7}{16}$, tarsus in ♂ 0·67, tarsus in ♀ 0·61.

16. **Geotrygon montana** (Linn.). ♂ "Perdrix rouge," ♀ "Perdrix noire." Pat. and Fr. (Red Partridge, Black Partridge.).

Rather common in heavy woods, particularly near the streams and rivers. Found mainly among the mountains. Like the "Ramier" it is much hunted as a game bird and is consequently shy. The native name of "Perdrix" (Partridge) is very appropriate, as it is very terrestrial in its habits, spending most of its time on the ground, frequently running instead of flying to avoid danger, and in many of its habits and general appearance it more resembles the quails or partridges than the doves.

It is a very quiet bird, rarely giving utterance to any sound. The dark colored (perhaps immature), female is much less frequently shot or seen than her more brilliantly colored mate.* Of the seven specimens in our collection only one is a female and dark brown, while the six males are all in the red plumage. Like the last it is frequently kept in captivity as a cage-bird.

Iris of the male varies from orange brown to yellow brown or ocher yellow, in the female it is yellow. Legs, feet, bill and naked skin surrounding the eye, pink in the male; darker red in the female when in the brown plumage. ♂ $11\frac{1}{2}$–$6\frac{1}{8}$–$3\frac{1}{4}$–$19\frac{3}{8}$; $11\frac{1}{4}$–6–$3\frac{3}{4}$–$19\frac{1}{4}$. ♀ $11\frac{1}{2}$–6–3.

"Nest built on or near the ground, generally of a few leaves loosely put together Eggs slightly tinged with buff, resembling those of *Bonasa umbellus*."—(A. H. V.)

* The adult female has been described as like the male, and the dark-brown plumage given as that of the young (Ridgway, Man. N. A. Birds), but natives say that the female is always brown, and our small series seems to bear out this assertion, for though I looked particularly for a red female or a brown male, I never found either. Mr. Cory, too, describes the sexes as different in his "Birds of the West Indies" (Auk, vol. iv, p. 119).

17. **Geotrygon mystacea** (Temm). "Perdrix keesong," Pat. (probably from Fr. Perdrix croissant, Crescent Partridge.).

The inhabitants told us of a "perdrix" called by the above Patois name and stated that it was found in much the same localities and had habits very similar to the former species but was much rarer.

Before I arrived one was shown to my brother, by Mr. Hennessey Dupigny I think, but it was so badly shot and injured that my brother, not realizing at the time its rarity on the island, did not preserve it. Though I saw no specimens myself, I have no doubt from the descriptions of my brother and Mr. Dupigny that the Dominican "Perdrix keesong" is the above species, recorded from both Guadeloupe and Martinique by Dr. L'Herminier, and taken on the former island only, by Mr. Ober, and according to him, there called "Perdrix croissant" from the white crescent-shaped mark under the eye. Also given from St. Lucia and Guadeloupe by Mr. Cory (Auk, vol. iv, p. 118), and taken at Martinique by Mr. W. B. Richardson (ib. p. 96).

## Order, RAPTORES.

### Family, FALCONIDÆ.

18. **Buteo latissimus** (Wils.). "Malfeenee," Pat.
*Buteo pennsylvanicus* (Wils.); Lawr. and Scl. Lists.

Common, widely distributed, and much more tame and unsuspicious than in the United States. We found it particularly common in the vicinity of Bass-en-ville. Observed from the time we arrived till we left. In several of the specimens taken, the stomach contained nothing but large caterpillars. All our specimens, six in number, were in the fully adult plumage.

Sexes alike. Iris white. ♂ 15½–10⅛–6¾–32. ♀ 15¼–10½–6½–33; 15½–10⅜–6½–33. In all specimens; bill, cere to tip, ¾; tarsus 2.

19. **Falco columbarius** Linn. "Nonnette," Pat. and Fr. (Osprey); "Gue Gue," Pat.; "Killee Killee," Pat.; "Mountain Hawk," Eng.

Rather rare but generally very tame. Neither Mr. Ober nor Mr. Ramage took this bird in Dominica, nor is it mentioned at all in Mr. Lawrence's List of the Birds observed among the Lesser Antilles by Mr. Ober, and Mr. Taylor does not mention seeing it anywhere among the West Indies, all of which seems rather strange, as we took two specimens, a pair (Mar. 7 and April 12), and saw others, and my brother found its nest, proving it to be a resident, at least during the breeding season. Dr. L'Herminier, however, gives it

from both Guadeloupe and Martinique, it was also taken in the latter island, by Mr. Richardson (Auk, iv, p. 96), and at St. Kitts by Mr. C. S. Winch (ib. viii, p. 48) and recorded from the Greater Antilles and Grenada by Mr. Cory (ib. iv, p. 43).

Apparently it is mainly confined to the mountains, as all we observed were seen at quite an altitude, and the common English name is "Mountain Hawk," while the other names, with the exception of the first, are also applied to the following species. I am at a loss to account for the name "Nonnette" (Osprey) as applied to this bird.

Iris (in specimens taken), brown in the male, red in the female; legs and feet yellow; bill dark horn-color, lighter at the base. ♂ 11½–7⅜–5. ♀ 11½–7½–5.

"Eggs all badly incubated. Breeds in hollow trees or on cliffs."—(A. H. V.)

20. **Falco caribbæarum** Gmel.. "Gue Gue," Pat.; "Killee Killee," Pat.
*Tinnunculus sparverius antillarum* (Gmel.); Lawr. List.
*Tinnunculus caribbæarum* (Gmel.); Scl. List.

Rather common, but not abundant. It much resembles *F. sparverius* in its habits and notes. It is a rather peculiar fact that all the specimens obtained, four in number, were males. Taken from April 14 to May 24.

Irides brown; cere, eyelids, feet and tarsi, orange yellow; bill bluish grey at the base, black at the tip. ♂ 11–6½–5½–20; 11½–6½–5 3/16; 11½–6⅝–5 3/16. In all specimens, tarsus 1¼, bill (cere to tip), ½.

21. **Pandion haliaëtus carolinensis** (Gmel.). Fish Hawk.
*Pandion haliætus* (Linn.); Lawr. List.

Seen several times flying over.

## Family, STRIGIDÆ.

22. **Strix flammea nigrescens** Lawr. "Shawah," Pat. (probably from Fr. Chat-huant, Screech Owl); "Owl," Eng.

Rather rare. Like the European species, it is found principally around old deserted houses, in the church towers, and similar places, where it also breeds. This bird, like many of its family elsewhere, is regarded with superstition by the natives and looked on as a "Jumbie Bird," or one possessed of evil spirits.

Sexes unlike. Irides dull, yellowish brown; feet and legs dark brown; feet and toes very sparsely feathered; bill very light yellow.

♂ (from skin), wing 9¼, tail 4¼, tarsus 2.

## Order, PSITTACI.

### Family, PSITTACIDÆ.

23. **Amazona augusta** (Vig.). "Ciceroo," Ca.

*Chrysotis augusta* (Vig.); Lawr. and Scl. Lists.

Common in the interior and on the windward side of the island where it is found among the mountains at quite an elevation. This beautiful bird inhabits the thickest and most impenetrable forests where the mountain palms and gommier trees grow, the seeds and fruit of which, together with the young shoots of the former, make a large part of its diet.

They are much hunted for food during the time the game law is off, being then occasionally for sale in the market at Roseau, consequently, though common in the particular localities where they live, they are exceeding shy and difficult to procure. Even when within gunshot it is very hard to distinguish their green and purple plumage among the dense foliage of the high trees. They are generally found in small flocks or in pairs and are not infrequently seen flying over at a distance. As Mr. Ober has observed, their calls are heard mainly for a short time in the early morning and towards evening, and when disturbed, as by a gunshot. Their notes are mainly a shrill whistle, a sharp scream, and a series of crescendo yells.

When kept in captivity, as they sometimes are, they make fairly good talkers. According to the natives, the nest is generally built in a hole at the top of a dead palm, but it is very rarely found; one old Carib, a man who had spent his life in the woods, said he had never found but one.

It was mainly to procure these truly Imperial Parrots, so seldom seen in collections, that our trip was made to Bass-en-ville, which is a single house in the primeval forest, and only to be reached by one of the worst trails I have ever traveled, and I have spent a number of months among the Sierra Nevada Mountains. This trip, however, well repaid us for our trouble, as it was there that we took many of our best birds and other specimens, but though parrots were seen nearly every day, and we were accompanied by Mr. Hennessey Dupigny and another hunter, our united efforts secured but two of these shy birds in the ten days we were there.

One of our specimens is a female and the other was so mutilated by a shot as to render the determination of sex impossible, but they are alike in plumage and I can see no difference between them and Mr. Lawrence's description of a male taken by Mr. Ober.

Iris red; bill dark horn-color with a light spot on each side of the upper mandible at the base; legs and feet dark brown. ♀ 21–11¼–8¼–36½; sex undetermined, 19⅔–10½–7½–35½.

24. **Amazona bouqueti** (Wagl.). "Perroquet," Pat. and Fr. (Parrot).
*Parrot* sp.?; Lawr. List.
*Chrysotis nichollsi* Lawr.; Proc. U. S. Nat. Mus. III, p. 254, 1880.
*Chrysotis bouqueti* (Wagl.); Scl. List.

Found in the same localities, but apparently much rarer than the "Ciceroo" and like it very wild and difficult to procure.

Generally seen in rather larger flocks, otherwise the remarks with regard to the habits and notes of *C. augusta* apply equally well to this species. The notes of the two species can generally be distinguished by a person familiar with them, but the difference between the yells of two parrots is something very difficult to indicate on paper. These latter are also said never to become good talkers.

We succeeded in procuring but one specimen, a male. Mr. Ober did not take this bird at all, though he mentions a parrot "about the size of our North Carolina Parrot but more robust"; but in 1879 and 1880 Dr. Nicholls sent two small collections of Dominican birds to the Smithsonian, and among them were three specimens that Mr. Lawrence described as *C. nichollsi*, giving several differences between it and *C. cyanopis* (Vieill.) and *C. bouqueti* (Bechat). Our specimen agrees very closely with his description in all points but one. Mr. Lawrence says "the breast and abdomen are tinged with yellow" and mentions this as one of the differences between *C. nichollsi* and *C. bouqueti*, but in our specimen the abdomen is green with many of the feathers dull red at the base and there is a band of scarlet ¾ of an inch wide, extending across the upper part of the breast, and half way around to the back on each side, and below the band, in the middle of the breast, are two or three scarlet and yellow feathers. In the specimens at the Am. Mus. of Nat. Hist. this band is entirely lacking. Mr. Cory, however, in his "Birds of West Indies," 1889, p. 186, mentions a "patch of dull red mixed with yellow on the upper part of the breast joining the throat"; but our specimen differs from his description in having the red, wing speculum covering three instead of two feathers and in a few other points and he does not mention the red on the base of the abdominal feathers nor the dark blue on the outer webs of the primaries and outer tail feather, so that in some respects our bird more resembles his description of *C. versicolor* (Müll.).

Judging from the various descriptions and our own specimen this species is subject to great variation in plumage, perhaps due partly to sex, age, and season.

Iris orange; bill light horn-color, running into black at tip of upper mandible; legs and feet brown. ♂ (from skin), wing 9½, tail 6, bill (chord of culmen from cere) 1.20.

## Order, COCCYGES.

### Family, CUCULIDÆ.

25. **Coccyzus minor** (Gmel.). "Coulaveecou," Pat.; "Coucoumioc," Pat. (Fr. Coucou manioc, Manioc or Cassava, Cuckoo).
*Coccyzus seniculus* Vieill.; Tayl. List.

Not uncommon, locally quite common, as at Bass-en-ville, where a number were seen and heard and several taken.

In habits and notes it much resembles our common Yellow-billed Cuckoo (*C. americanus*).

Iris brown or red; upper mandible black, lower mandible yellow, black at the tip; legs and feet black. Sexes alike in plumage, female apparently larger than the male. ♂ 13–5⅜–6⅝–15; 13½–5⅜–6¼; 12¾–5¼–6–16½. ♀ 13¾–5¾–6⅞–17½.

## Order, ALCYONES.

### Family, ALCEDINIDÆ.

26. **Ceryle alcyon** (Linn.). "Calbasco," Pat.

Rather rare and shy. Seen several times. Though no specimens were obtained it was undoubtedly this species, which Mr. Ober also saw but did not take.

The natives insisted that there were two entirely different birds, both called by the above Patois name, and stated that the other was rare and had a long bill with teeth or notches along its sides, but the description was so imperfect that it was impossible to tell what bird it was, and though we used every endeavor and offered a good price we were unable to procure any; still I am in hopes that one of our friends who helped us to secure so many rare birds, may yet send us one of these, which I am sure from the description given us is something unlooked for in the fauna of this island.

## Order, MACROCHIRES.

### Family, MICROPODIDÆ.

27. **Cypseloides niger** (Gmel.). "Hirondelle," Pat. and Fr. (Swallow).

Not so common as the following species, though generally seen in the same localities and at the same time, but usually flying much higher, and so is more difficult to procure. Two specimens only were obtained. Neither Mr. Ober nor Mr. Ramage procured this bird in Dominica, though the former gentleman speaks of seeing "a species of swift intermediate in size between the small swift and the large martin," which was undoubtedly this bird.

Iris, bill, legs, and feet black. Sex? $6\frac{3}{4}$–6–$2\frac{5}{8}$.

28. **Chætura dominicana** Lawr. "Hirondelle," Pat. and Fr.

Given provisionally as *Chætura poliura* (Temm.) by Mr. Lawrence in his list. Afterwards described as above. (Ann. N. Y. Acad. Sci., vol. i, 1879, p. 255.)

Very common and widely distributed. Particularly abundant near Spring Hill and about the head of the Roseau Valley. Also seen in numbers at Casata Garden and at Bass-en-ville. As Mr. Ober states, they generally appear for a short time after a rain and then disappear again. I can see no trace in our specimens of the whitish edging to the upper tail coverts mentioned by Mr. Lawrence. Very likely it is a seasonal variation.

Sexes alike. Iris and bill black, legs and feet brown. ♂ 5–$4\frac{5}{16}$–$1\frac{3}{4}$–$10\frac{1}{4}$; $4\frac{3}{4}$–$4\frac{5}{8}$–$1\frac{3}{4}$–$10\frac{1}{2}$. ♀ $4\frac{3}{4}$–$4\frac{7}{16}$–$1\frac{5}{8}$–$10\frac{3}{4}$; $4\frac{3}{4}$–$4\frac{7}{16}$–$1\frac{3}{4}$–$10\frac{7}{8}$; $4\frac{3}{4}$–$4\frac{5}{8}$–$1\frac{5}{8}$. Tarsus $\frac{3}{8}$ in all specimens.

### Family, TROCHILIDÆ.

29. **Eulampis jugularis** (Linn.) "Fou Fou Mardet," Pat. (Fr. Fou Fou, Crazy Crazy, in allusion to their eccentric motions while on the wing.)

Common, though rarely found in the immediate vicinity of the coast. Its principal range seems to be from 500–1500 feet elevation and in this belt it is very common, especially in the plantain, banana, and lime plantations where it may be seen hovering about the flowers, perching every now and then on some convenient leaf or twig to rest and preen itself. Like all of its family that are found on the island, it is very tame and permits a close approach.

Formerly many of this and other species were slaughtered for millinery purposes, but of late years they have been strictly protected by law. Mr. Taylor speaks of finding this bird in Dominica, but less commonly than the following species, whereas our experi-

ence was directly the reverse, this species outnumbering *E. holosericeus*, I should say, by at least four to one, and closely approaching *B. exilis* in numbers, so that it may have increased since he was there. He also speaks of its frequenting "thick shady places," a habit that we failed to observe.

The females closely resemble the males but the colors are not quite so bright, particularly the crimson on the throat and breast, and they seem to be slightly smaller. The bill (exposed culmen), varies greatly in length. Iris, legs, feet, and bill black. ♂ $5\frac{1}{2}$–$3\frac{1}{8}$–$1\frac{3}{8}$–$7\frac{5}{8}$, bill ·92; $5\frac{1}{4}$–3–$1\frac{5}{8}$–$7\frac{1}{2}$, bill ·85 ; $5\frac{1}{8}$–$2\frac{7}{8}$–$1\frac{5}{8}$, bill 1·03. ♀ $5\frac{3}{8}$–$2\frac{13}{16}$–$1\frac{1}{2}$–7, bill 1·04 ; 5–$2\frac{3}{4}$–$1\frac{9}{16}$, bill ·97 ; 5–$3\frac{1}{8}$–$1\frac{7}{8}$, bill ·85.

"Nest always placed at a considerable distance from the ground. One taken April 9th was fully sixty feet from the ground in a catalpa tree."—(A. H. V.)

This nest, though entirely finished, contained no eggs and evidently had not been used. It is saddled on a good sized crotch (the main branch $\frac{5}{16}$ inch in diameter), that grew in a nearly horizontal position. The nest is very compact, slightly elliptical in shape on top, and composed of the brown scales from the leaves of ferns, probably mainly tree-ferns, with a few large pieces of grey lichens on the outside, mostly near the bottom. The bottom is covered with greenish white down, probably from young fern leaves, and the whole nest is very firmly fastened to the branch by the same material, running entirely around the twig. Inside, it is lined with down from the silk-cotton tree. From side to side, in the largest place, it measures $2\frac{1}{8} \times 1\frac{3}{4}$ and is $1\frac{3}{4}$ high. The cavity is $1\frac{1}{8} \times 1$ on top and $\frac{5}{8}$ deep. Plate xxv, fig. 1.

30. **Eulampis holosericeus** (Linn.) "Fou Fou Tête-longue." Pat. and Fr. (Crazy Crazy Long-head.)

Not so common as the preceding, found at a rather greater elevation (about 750–2000 feet), and principally on the windward side of the island, though by no means rare on the leeward side in certain localities. In common with the last and *B. exilis* it is particularly fond of the plantain and banana patches, which were our principal collecting grounds for these three species.

Mr. Taylor states that he found this the most abundant hummingbird in Dominica, but according to our experience it was the least so, with the exception of *T. bicolor*, which was not observed by him, so that it seems as though this species must have greatly decreased or the others increased since he was there, which well might happen in the twenty-seven years between his visit and ours.

Sexes much alike, but the male rather brighter than his mate. Iris, bill, leg, and feet black. As in the last species the length of the bill is very variable. The measurement given in all cases is the chord of the exposed culmen. ♂ $4\frac{3}{4}$–$2\frac{1}{4}$–$1\frac{7}{16}$–$5\frac{1}{2}$, bill ·80; $4\frac{3}{4}$–$2\frac{7}{16}$–$1\frac{7}{16}$, bill ·82; $4\frac{1}{2}$–$2\frac{5}{16}$–$1\frac{1}{2}$, bill ·96. ♀ $4\frac{3}{4}$–$2\frac{3}{8}$–$1\frac{7}{16}$–6, bill 1.01.

"Nest generally a deep pouch-shaped structure fastened by one side to a perpendicular twig, generally at some height (20–40 feet), from the ground."—(A. H. V.)

Five nests are in the collection.

One taken at Laudat, April 10, contained no eggs, is pouch-shaped, fastened to the upright stalk of a plant, and is composed of the fine, brown scales from ferns, mentioned above, with a number of pieces of bark and leaves, from the plantain and banana plants, hung loosely on the outside, the whole being bound together by cobweb. It is $2\frac{1}{2}$ high and $1\frac{1}{2}$ in diameter at the top, continuing the same size to within an inch of the bottom and from there tapering down to a point. The cavity is $1\frac{3}{8}$ deep and $\frac{3}{4}$ in diameter.

The second was taken March 28, and contained two young. This is like the last except that it is completely covered with pieces of plantain or banana bark and leaves. It is $2\frac{1}{2}$ high and $1\frac{1}{2}$ in diameter across the top, sloping gradually down to the bottom, which is $\frac{3}{4}$ across. The cavity is $1\frac{1}{8}$ deep and 1 in diameter across the top. Plate XXVI, fig. 2.

The third, taken April 13th, contained two fresh eggs. It is cup-shaped, saddled on a small twig inclined upwards at an angle of about 30°, and is composed like the two already described. It measures $1\frac{1}{2}$ across the top and is $1\frac{1}{4}$ high. The cavity is $\frac{7}{8}$ across the top and $\frac{3}{4}$ deep. The eggs are dead white, elliptical in shape, and measure ·51 × ·34 and ·50 × ·35. Plate XXV, fig. 2.

The fourth, taken April 15th, also contained two fresh eggs. It is cup-shaped and composed like the former ones but with a little down from the silk-cotton tree for inside lining and very few pieces of banana and plantain leaves on the outside. It is $2\frac{1}{2}$ high and $1\frac{1}{2}$ across the top, sloping down to a point at the bottom. The cavity is $\frac{3}{4}$ deep and $\frac{7}{8}$ in diameter. The eggs are like the above and measure ·46 × ·29 and ·47 × 30.

The last nest contains two eggs. It is cup-shaped and composed like the third. It measures $1\frac{1}{4}$ high and $1\frac{5}{8}$ across the top. The cavity is $\frac{3}{4}$ deep and 1 across the top. The eggs measure ·51 × ·33 and ·51 × ·34, color and shape like the former ones. Plate XXVI, fig. 1.

31. **Thalurania bicolor** (Gmel.) "Fou Fou Bleu," Pat. and Fr. (Blue Crazy Crazy).

*Thalurania wagleri* (Less.); Lawr. and Scl. Lists.

Rather common. Found mainly at a considerable elevation and in the heavy woods. We never took it at an elevation of less than 1200 feet and apparently it rarely, if ever, descends to the lowlands. Taken in the vicinity of Laudat, at Providence (Mr. Hennessey Dupigny's plantation near Laudat), and near Bass-en-ville. This beautiful bird seems to prefer the seclusion and shade of the deep woods to the sunshine and warmth of the plantations and clearings. In some little opening among the thick mass of trees, vines, lianas, etc., that compose the forests of this island or along some mountain trail that runs through the deep woods, it is generally seen, either hovering about some flower, sitting pruning itself upon some twig or stem, or darting past so swiftly that one sees but a gleam of burnished blue and green. It is rather solitary in its habits, more than one rarely being seen at a time. Only one female was obtained, at Bass-en-ville, May 19, and no others were met with, though nine males were obtained and several others seen. The males too were observed about the nests and sitting on the eggs, but no females.

Sexes very different in plumage. Iris dark brown or black; legs and feet dark brown; upper mandible black; in the male the lower mandible is white, black at the tip; in the female it is dark brown, slightly lighter at the base and black at the tip. ♂ $4-2\frac{1}{4}-1\frac{9}{16}$; $4\frac{1}{2}-2\frac{3}{8}-1\frac{5}{8}-6$; $4\frac{3}{8}-2\frac{5}{16}-1\frac{11}{16}-5\frac{3}{4}$; $4\frac{1}{2}-2\frac{5}{16}-1\frac{5}{8}-6$; $4\frac{1}{4}-2\frac{3}{8}-1\frac{3}{4}$. ♀ $4-2\frac{3}{16}-1\frac{1}{2}-5\frac{1}{2}$. In all the male specimens the exposed culmen measures 0·65 and in the female it is 0·68.

"This bird builds the handsomest nests of all the hummers on the island. It is usually built close to the ground, never more than a few feet from it, and generally placed in a large brake-like fern. A number of sets taken, all at a great elevation, 2000-2500 feet."—(A. H. V.)

One nest, taken April 12, contained "two badly incubated eggs." It is fastened to the frond of a fern, and composed mainly of a fine, short, greyish brown, vegetable fibre, much resembling fur, and a few brown fern scales. On the outside are fastened a few small pieces of gray lichen and the whole is fastened together with spider-web or something so closely resembling it as to be indistinguishable without careful microscopic examination. It measures $1\frac{3}{8}$ across the top, $1\frac{1}{4}$ in height, and the cavity is $\frac{3}{4}$ deep. The eggs are dead white, nearly elliptical, and measure ·46×·30 and ·46×·31. Plate XXVI, fig. 5.

Another nest, taken April 14, containing one fresh egg, is situated on a twig and much like the last, but slightly lined with silk-cotton, and is well covered with small pieces of lichen, each placed with the natural side, of a light greyish green, out. It is $1\frac{3}{8}$ across the top, $1\frac{1}{8}$ high, and the cavity is $\frac{7}{8}$ deep. The egg is like the last two and measures ·54 × ·37. Plate XXVI, fig. 4.

A third nest taken near Laudat, March 28, with two badly incubated eggs, is placed in the crotch of a fern frond, but varies from the other two in being composed, on the outside, of the brown scales from stems of ferns with no pieces of lichen, and the inside is very thickly lined with down from the silk-cotton tree. This one measures $1\frac{1}{2}$ across the top, $1\frac{1}{4}$ in height, and the depth of cavity is $\frac{7}{8}$. One egg is too badly broken to measure, the other is ·49 × ·32.

32. **Bellona exilis** (Gmel.) "Fou Fou Bequar," Pat. (Possibly from Fr. besquée, a beak full.)

*Orthorhynchus exilis* (Gmel.); Tayl. Lawr., and Scl. List.

Very common and widely distributed. Though most abundant in the low lands and near the coast, it is also found commonly among the mountains and we took it from every camp. According to our experience this pretty and diminutive species is the commonest of the hummers in Dominica.

Sexes unlike. ♂ $3\frac{1}{2}$–$1\frac{7}{8}$–$1\frac{3}{16}$, exposed culmen ·31, bill (from rictus) ·68. ♀ $3\frac{1}{2}$–$1\frac{13}{16}$–$1\frac{3}{16}$, exposed culmen ·43, bill (from rictus) ·70; $3\frac{1}{2}$–$1\frac{3}{4}$–$1\frac{1}{4}$, exposed culmen ·44, bill (from rictus) ·67.

The nests of this species vary considerably in composition, shape, and mode of attachment. There are nine nests in the collection, no two just alike, the only characters common to all seem to be the presence of more or less brown scales from ferns or tree-ferns used in the composition, and the use of spider web, or some very similar material, in greater or less quantity, to bind the nest together.

Generally the nest is composed mainly of the fern scales, with more or less silk-cotton for a lining, it being entirely absent in only two out of the nine nests, and in three it constitutes about half the total bulk of material. In a number it is also used on the outside, in small quantities, to help bind them together. Six nests are more or less ornamented on the outside with pieces of lichen, after the manner of *T. bicolor*. In one of the other nests the lichen is replaced by small pieces of brown bark and in one where lichen is present there are also a few pieces of dark green moss. (Plate XXVI, fig. 6).

The size is pretty constant but the shape varies more or less according to the location. The usual form is cup-shaped or some modification of it. When saddled on a horizontal twig the sides are generally straight and when in a crotch or on an upright they generally slope somewhat. The diameter across the top is about 1¼, three are 1⅜; the height varies from 1⅛ to 1⅝, with the exception of one particularly shallow one that is only ¾ high. The cavity varies in depth from ½ to ⅞, the average being about ⅝, and in diameter across the top, from ¾ to ⅞.

The mode of attachment and location is quite various. Two are saddled on horizontal branches, one of them at the junction of two smaller twigs; two more are on twigs inclined upwards at an angle of about 45°, one at the branching tip and the other at a crotch; one is in the crotch of a fern leaf, a very pretty and compact nest, thickly covered with spider-web and lined with silk-cotton (Plate XXVI, fig. 3.); another is fastened by the side to two twigs near their junction with a larger branch; another is fastened to the stem of a drooping leaf; and still another by its side to the stem of a vine at the junction of two leaves.

The eggs are dead white and elliptical in shape, like those of the other hummers. We never found more than two in a set. One set of two, taken April 13, were slightly incubated and measure ·50×·32 and 51×·32; another, taken three days later, had only one egg, fresh, measuring ·47×·32; and on March 14 another nest contained two badly incubated eggs, one only saved, ·47×·31; and still another taken more than a month later, April 19, also contained two eggs badly incubated, so that only one was saved, measuring ·45×·31. Evidently the breeding season varies much.

## Order, PASSERES.

### Family, TYRANNIDÆ.

**33. Tyrannus rostratus** Scl. "Pipiree," Pat. (from the note).

Common locally. We found it quite plentiful at Bass-en-ville and all up and down the Layou Valley, but rare in the Roseau Valley. A very noisy and lively, but suspicious bird. It delights to perch on the top of some dead tree and utter its loud cry of "pipiree, pipiree," every now and again darting into the air after some passing insect. Its general habits much resemble those of our common Kingbird (*T. tyrannus*).

Sexes much alike. Iris brown; bill, legs, and feet black. ♂ 10–5–3½–15; 10–5–4–15; 10–4¾–4–15½. ♀ 9½–4½–3½–13½; 9⅝–4½–3⅞–15.

34. **Myiarchus tyrannulus oberi** (Lawr.) "La belle Gotet," Pat. (Fr. Gros tête, Large head). "Soleil Coucher," Pat. and Fr. "Sunset Bird," Eng.

*Myiarchus*, sp. ?; Tayl. List. Afterwards referred to *M. erythrocercus.*
*Myiarchus oberi* Lawr.; Lawr. List.
*Myiarchus tyrannulus* (Müller).; Scl. List.

Rather common in certain localities, as at Bass-en-ville, but generally very shy and suspicious. Mainly confined to the uplands and mountains. General habits and note somewhat resemble those of *M. crinitus.* The names "Soleil Coucher" and "Sunset Bird" come from its uttering its cry at about sunset.

Sexes alike in plumage, female apparently slightly smaller than the male. Iris dark brown; bill, legs and feet black. ♂ $9\frac{1}{8}$–4–$3\frac{3}{4}$–$13\frac{1}{4}$; 9–4–$3\frac{1}{2}$–12. ♀ 9–4–$3\frac{5}{8}$–12; $8\frac{7}{8}$–$3\frac{3}{4}$–$3\frac{3}{8}$–$12\frac{3}{4}$. 8–$3\frac{3}{4}$–$3\frac{3}{8}$; 8–$3\frac{5}{8}$–$3\frac{1}{4}$.

In all our specimens the bright ferruginous extends along the entire length of the inner webs of the tail feathers, instead of being confined to their outer two-thirds as given by Mr. Lawrence in his original description (Ann. N. Y. Acad. Sci., vol. i, p. 48).

The color of the ventral surface is very variable, grading from light, but bright and pure, yellow in some to dull white, merely washed or tinged with yellow, in others. This variation is apparently not due to sex, season, or locality, as two males taken within three days of each other, at the same place, show as much variation as any.

35. **Blacicus brunneicapillus** Lawr. "Gobe-mouche," Pat. and Fr. (Fly-catcher).

Common, particularly abundant in the heavy woods fringing the main road up the Roseau Valley. An inhabitant of the mountains rather than the lowlands, seeming to generally prefer the deep, high, woods and their borders to the more open groves and plantations of less elevated parts of the country.

Sexes similar. Irides dark brown, legs and feet brownish black, upper mandible black, lower mandible light yellow. ♂ $6\frac{1}{2}$–$2\frac{3}{4}$–$2\frac{3}{4}$–$8\frac{1}{2}$; $6\frac{1}{4}$–$2\frac{11}{16}$–$2\frac{5}{8}$–$9\frac{1}{4}$; 6–$2\frac{11}{16}$–$2\frac{5}{8}$–$8\frac{1}{2}$; 6–$2\frac{9}{16}$–$2\frac{9}{16}$. ♀ 6–$2\frac{3}{4}$–$2\frac{11}{16}$; $5\frac{7}{8}$–$2\frac{9}{16}$–$2\frac{1}{2}$–$8\frac{1}{4}$. In all specimens, bill $\frac{7}{16}$, tarsus $\frac{9}{16}$ to $\frac{5}{8}$.

The only nest obtained was taken with two fresh eggs, April 16. It was placed on a dead limb and is a very frail, loose structure of bark, pieces of banana or plantain leaves, stems, and roots, 2 inches in diameter and $\frac{3}{4}$ high.

The eggs are white with a ring of confluent spots and blotches of a deep reddish brown and a few of lilac, about the large end, in one

egg completely covering this end; the other is not so heavily marked and has a few small spots and blotches over the rest of the surface. They measure ·74×·56 and .76×·55. Plate xxv, figs. 5 and 6.

36. **Elænea pagana martinica** (Linn.). "Cheweck," Pat. (from the note)
*Elainea martinica* (Linn.); Tayl., Lawr., and Scl. Lists.

Common. Generally found at quite an elevation. In habits it much resembles our Pewee (*Sayornis phœbe*).

Sexes alike in plumage. Iris dark brown; upper mandible dark horn-color, lower mandible yellow, running into dark horn at the tip; legs and feet black. ♂ 6⅞–3¼–3–10⅝; 7–3⅛–2¾. ♀ 6¾–3⅛–2½–10; 7–3⅛–2¾.

"Nest resembles that of *Empidonax minimus*. Eggs very variable. Generally white, spotted about the larger end with lilac and brown. Sometimes closely resembling those of the 'Teeteen' but larger."—(A. H. V.)

A nest in the collection, taken April 16th, contained three fresh eggs. It is compactly built of dry stems, fine grass, and vegetable fibre and is lined with the same and some down from the silk-cotton tree. It measures 2½ in. across the top and 1¾ in. high. The cavity is 1⅝ across the top and 1¼ deep.

The eggs are white with fine, light sepia-brown and grey spots (the brown greatly predominating), confluent, and forming a ring about, and even in one case covering, the large end. On the rest of the egg the spots are much lighter. Taken as a whole the markings are more in number and finer than in *D. petechia melanoptera*. The eggs measure ·68×·49; ·68×·48; ·68×·51. Plate xxv, figs. 7 and 8.

## Family, Fringillidæ.

37. **Loxigilla noctis schlateri** Allen. ♂ "Penwe," ♀ "Masong," Pat. (Probably from Fr. Père noir, Black father, and Mère sang, Blood-colored mother in allusion to the black male and rather rusty colored female). Also called "Robin" and "Plantain-eater."
*Loxigilla noctis* (Linn.); Tayl., Lawr., and Scl. Lists.

Very abundant, especially in the valleys and about the plantations and towns. These were generally the first birds to respond to the call made by sucking the back of the hand, and invariably manifested great anxiety and excitement. The sexes are usually very different but subject to great variation. In this connection Mr. Allen, who made a careful examination of our series, writes: "Three males present much variation in the amount of rufous on the lower tail-coverts and the size of the supra-loral spot. These, with other Dominican specimens before me, show that the rufous may be en-

tirely lacking from the lower tail-coverts, or occupy them to the exclusion of black. Two of the females have rufous feathers on the throat, forming, in one of them a small patch; in this specimen there is more or less black mixed through the plumage, giving a patchy effect, suggesting a young male in changing plumage. The sex, however, was carefully determined by dissection. [This specimen also agrees with the other females in size.—G. E. V.] It thus appears that the female sometimes partially assumes the livery of the male, as Mr. Cory has recently found is the case in *Loxigilla violacea* (cf. Auk, viii., 1891, p. 296)."

Irides red or brown; upper mandible black or dark horn, lower mandible lighter, particularly in the female; legs and feet brown. ♂ 6–2⅞–2⅛; 5½–2⅞–2⅛; 5⅞–2⅞–2–9½. ♀ 5½–2⅝–2–8½; 5½–2¾–2⅛.

"Nest a bulky affair of leaves, sticks, etc. Sometimes domed with the opening on one side. Breeds anywhere. Eggs two to five. A number of sets taken March 28–April 9."—(A. H. V.)

There are two nests of this species in the collection, neither of them domed. One, taken April 1, is composed of pieces of dry plantain and banana leaves, fine stems, roots and dead leaves, and is lined with dried grass, stems, etc. It measures 3 in height and 3¾ across the top. The cavity is 1⅝ deep. This nest contained two fresh eggs, white, blotched and spotted with reddish brown, confluent at the large end, and measuring ·81 × ·57 and ·84 × ·61.

Another nest taken March 31 contained one fresh egg. The nest is like the last and about the same size. The egg markings are of very light brown, not so heavy as in the last, and the large end is not so heavily ringed. It measures ·80 × ·58. On another egg, from a third set, the spots are much more even, rather darker, and form no ring at the large end. This egg measures ·85 × ·58.

38. **Euetheia bicolor** (Linn.) ♂ "Zee Zee Zeb." ♀ "Zee Zee Zay" (Pat.).

*Phonipara bicolor* (Linn.): Lawr., and Scl. Lists.

*Phonipara omissa* Jard.; Tayl. List.

Abundant, but like the last, found principally in the neighborhood of plantations and along the trails and paths. Not often seen in the interior nor far from the settlements and cleared lands.

"Note resembles that of the Yellow-winged Sparrow (*Ammodramus savannarum passerinus*)."—(A. H. V.)

Sexes different in plumage. Iris black, legs and feet dark brown, bill black. ♂ 4½–2–1⅝–6½; 4¼–2–1½–6 1/16; 4–2–1⅝. ♀ 4½–2–1½–6½; 4½–2–1⅜.

"Nest built of grass, near the ground, among tall weeds or in the cane-fields. Entrance on the side."—(A. H. V.)

The two eggs in the collection are light greenish or dirty white with a number of dark brown spots at the large end and few of the same color scattered over the rest of the egg. They measure ·61 × ·48.

Family, TANAGRIDÆ.

39. **Euphonia flavifrons** (Sparrm.). "Peritch," Pat. (Fr. Perruch, Parroquet). "Jaco," Pat. and Fr. (Parrot).

Rare. We obtained all our specimens, nine in number, from the same tree and saw no others anywhere else. At this particular tree, however, one or more could almost always be obtained, at least by waiting a short time. The natives insisted that there was only one other tree of the same kind on the island and that these two trees were the only places where the birds were found. The tree in question had a parasitic vine of some sort growing on it and it appeared to be on the berries of this vine that the birds fed, as their crops were nearly always full of them.

These birds are very quiet, so that we heard them utter no note beyond a few chirps and twitters. In their actions they are slow and deliberate, crawling rather than hopping about, from which habit, probably, as well as their bright colors, the natives call them "Jaco" and "Peritch," apparently fancying they either are parrots themselves or bear some close relation to them.

The sexes are very similar, the colors of the male brighter than in the female but otherwise the same. Irides black, bill black, legs and feet greyish black. ♂ 5–2⅝–1⅝–8¼; 5–2½–1⅝. ♀ 5–2½–1⅝.

"Nest built of sticks, in a hole in a tree. Eggs pure white, sometimes slightly spotted. One nest found, but the eggs were badly incubated and not saved."—(A. H. V.)

40. **Saltator guadelupensis** Lafr. "Grosbec," Pat. and Fr. (Grosbeak).

Not common. A shy inhabitant of the thickest underbrush and bushes, generally found along the borders of the paths and cleared land. Sexes similar. Of the seasonal variation in this bird Mr. Allen, who examined our series, writes as follows: "This insular form of *S. albicollis* is represented by seven specimens, showing considerable variation in color, the March and April specimens being much greener, especially below, than those taken during the last half of May."

Iris brown; legs and feet brown; upper mandible dark horn-color at the base, yellow at the tip; lower mandible yellow with a large dark spot on each side at the base. ♂ 9–4⅛–3⅞–13½; 9–4–3½–12½; 9⅛–4–3⅞–13⅝; 8–3⅞–3½; 8–3¾–3⅝. ♀ 8¾–4⅛–3½–13; 8¾–4⅛–3½–13¼.

### Family, HIRUNDINIDÆ.

41. **Progne dominicensis** (Gmel.). "Hirondelle," Pat. and Fr. (Swallow).

A martin or large swallow was observed rather commonly about Roseau, but very rarely seen elsewhere. No specimens were taken, but it was undoubtedly the above species which was obtained by Mr. Ober.

### Family, VIREONIDÆ.

42. **Vireo calidris** (Linn.). "Cheweck Tetlong," Pat. (Fr. Tête longue. Long head).

*Vireosylvia altiloqua* (Vieill.); Tayl. List.
*Vireosylvia calidris* var. *dominicana* Lawr.; Lawr. List.
*Vireosylvia calidris* (Linn.); Scl. List.

Common and widely distributed. In habits and note resembling *V. olivaceus.* Very likely, as Mr. Ober thought, this bird is a summer visitor only, for our first specimen was not taken until March 27.

Sexes much alike. Iris generally brown, but in one specimen, a female, it was red; upper mandible dark horn, the lower bluish-white. ♂ $6\frac{1}{2}$–$3\frac{1}{4}$–$2\frac{1}{4}$–10; 7–$3\frac{1}{4}$–$2\frac{7}{16}$–10; $6\frac{1}{2}$–$3\frac{1}{8}$–$2\frac{5}{16}$. ♀ $6\frac{1}{4}$–3–2–$9\frac{3}{8}$; 6–$3\frac{1}{8}$–$2\frac{3}{16}$–9; $6\frac{5}{8}$–3–$2\frac{3}{16}$–$9\frac{3}{4}$. Exposed culmen in ♂ ·65, in ♀ ·60; tarsus in both sexes ·75.

### Family, CŒREBIDÆ.

43. **Cœreba dominicana** (Taylor). "Sucrier," Pat. and Fr. (Sugarmaker.)

*Certhiola dominicana* Taylor; Tayl., Lawr., and Scl. Lists.

Abundant almost everywhere. Sexes similar. One of the males (perhaps immature), differs from the others in having the anterior half of the superciliary line bright yellow, very little grey on the forehead, and the back greyish black instead of jet black as in the others. Bill black, legs and feet dark brown. They seem to vary much in size, the wings of the males ranging from $2\frac{1}{2}$–$2\frac{1}{8}$, the tails from $1\frac{3}{4}$–$1\frac{1}{4}$, the bills from ·53–·50 and the tarsi from ·74–·66. ♂ $4\frac{1}{2}$–$2\frac{1}{2}$–$1\frac{1}{2}$.

"Nest built almost anywhere, composed of a great variety of materials. Sometimes it is very beautiful and composed wholly of moss. Generally globular in shape with the opening on one side. Breeds continually from February to May."—(A. H. V.)

One nest in the collection was taken at Laudat, March 26th, and contained two slightly incubated eggs. It is globular in shape with the opening on one side and composed of leaves, grass, roots and stems. The mouth of the opening is lined with very fine rootlets

and stems. The total height of this nest is 5¾ in.; greatest diameter, from the bottom of the opening to back of nest outside, is 4¼ in. The opening is 1¼ in. high, 1 in. wide and situated 2½ in. from the bottom of the nest. (Plate xxv, fig. 3.) The eggs measure ·74×·52 and ·77×·52. The ground color is white very thickly and finely spotted with light chocolate-brown (in one so as to entirely obscure the white, except at the tip of the small end, which is clear white), heavier at the large end, where are a few fine lines of much darker brown.

Another nest is composed like the last, but with the outside beautifully covered with dark green moss like that in the nest of the "Morvy," (*Cichlherminia dominicensis*).

Another nest with four fresh eggs, taken April 10th, is composed like the first, but much smaller, measuring 3¼ in. in height and 3⅝ in. from front to back. The opening is round, 1 in. in diameter, and is 1½ in. from the bottom of the nest. The eggs measured ·69×·51, ·70×·51, and ·71×·53, the fourth is badly broken. The first resembles the former set but with the large end darker, Plate xxv, fig. 9; the second has around the large end a much darker band which is clearly defined toward the small end, but gradually grows a little lighter at the extreme large end, the rest of the egg is not so thickly marked as in the former examples, Plate xxv, fig. 10; in the third the spots are much larger, the large end is very thickly marked and the rest of the egg not very thickly. The last egg, which has been so broken as to prevent measuring, closely resembles the set first described.

Two other sets measure as follows: one set ·71×·49; ·74×·51; ·76×·52 and the other ·70×·49; ·70×·51; ·73×·51.

The color and markings are much like those previously described.

## Family, Mniotiltidæ.

44. **Dendroica petechia melanoptera** Lawr. "Teeteen," Pat.

*D. petechia* (Linn.); Tayl. and Lawr. List. Afterwards described by Lawrence as above (P. U. S. N. M., vol. i, p. 453).

*D. melanoptera* Sharpe; Scl. List.

Abundant in low, open woods, lime groves, and on the estates. Found low down, near the coast, as well as higher up in the mountains. Habits and notes much resemble *D. æstiva*. Sexes unlike in color. One female shows a faint trace of rufous on the crown and has a few light rufous streaks below. Irides dark brown, bill black, legs and feet light brown.

♂ 5–2⅜–1½–7; 5–2¼–2–7. ♀ 5–2⅜–1⅝–7; 5–2–1¾–7.

Several nests and sets of eggs are in the collection. Two characteristic ones, may be described as follows:

The first nest, taken on April 17, contained two fresh eggs, one ·64 × ·49, white with very slight greenish tint, with a ring of small brown and grey confluent spots about the larger end, the rest of the egg sparingly spotted with the same colors, thickest at the large end, very lightly toward the small end, the brown predominating over the whole egg. The other egg is ·65 × ·50, much like the other, but with the brown spots much larger, clearer, and of a deeper brown with no grey spots and fewer brown ones outside of the ring at the large end. Plate xxv, fig. 11.

The nest is compactly made of dry grass, stems of plants, leaves, chicken feathers, bits of cloth, colored wool, and several cocoons of spiders. It is lined with chicken feathers and horse hair, and measures 2¼ in. across the top and 2 in. high; the cavity is 1⅝ in. across the top and 1½ in. deep.

The other nest taken on April 9th contained three fresh eggs intermediate in color between the two just described and measuring ·65 × ·50, ·64 × ·49, and ·64 × ·50. The nest is much like the last but composed of more dried grasses and quite a little cotton, and contains no chicken feathers nor bits of cloth. It is lined with horse hair and a little down from the silk-cotton tree.

45. **Dendroica plumbea** Lawr. "Pa-pia," Pat.

PLATE XXVII, FIG. 2.

Very abundant, found nearly everywhere. Very tame and unsuspicious. Generally seen running up and down trunks of trees and hanging on the terminal twigs and leaves after the manner of the nuthatches and titmice.

Sexes alike. The green plumage described by Mr. Lawrence (Ann. N. Y. Acad. Sci., vol. i, 1879, p. 47), as that of the female is evidently the livery of the young as we took two females in the full grey plumage like that of the males, and one young female, April 14, with the grey feathers appearing on the top of the head, back of neck, around the bill, and on the throat, but otherwise agreeing with Mr. Lawrence's description of the female plumage, except that the line over the eye and the lower eyelid are both bright yellow instead of white as he describes them.

Iris dark brown; upper mandible dark horn-color; the lower yellowish, growing darker at the tip; legs and feet brownish yellow. ♂ 5½–2½–2–8; 5¾–2 9/16–2 5/16–8, tarsus 13/16. ♀ 5½–2 7/16–2⅛–7¾; 5½–2⅜–

$2\frac{1}{4}$; ♀ juv. $5\frac{1}{2}$–$2\frac{3}{8}$–$2\frac{1}{4}$. In all specimens the bill, from front, measures $\frac{7}{16}$, and the tarsus (except in one case), $\frac{3}{4}$.

"Nest very much like *Spinus tristis*, built in low shrubbery, particularly oleanders and grape plums. Eggs very much like *D. petechia melanoptera*, but generally larger and more heavily marked. Laudat, April 1; Shawford, April 19."—A. H. V.

46. **Seiurus noveboracensis** (Gmel.)

A bird described by the natives as being there early in the season, was doubtless this species, which was taken by Mr. Ober and which probably occurs simply as a migrant.

47. **Setophaga ruticilla** (Linn.) "Officer Bird," Eng. "Chat," Eng.

Probably a migrant only, as none were seen after May 1st. Sexes unlike. Differs in no way from New England specimens.

♂ 5–$2\frac{1}{2}$–$2\frac{3}{8}$; ♀ 5–$2\frac{3}{8}$–$2\frac{1}{4}$.

## Family, Troglodytidæ.

48. **Cinclocerthia ruficauda** (Gould). "Trembleur," Pat. and Fr. "Trembler" (Eng.).

Abundant and very widely distributed. Taken at every camp. Found chiefly in dense shrubbery near the ground, though frequently seen in the tops of tall trees searching for insects after the manner of the warblers and vireos. Towards evening it also ascends to the top of some bush or tree to sing, after the manner of the Brown Thrasher (*Harporhynchus rufus*), which it resembles in many of its habits and notes. Its name comes from its peculiar habit of constantly vibrating or "trembling" its wings, which are generally carried slightly raised from the sides and with the tips beneath the tail, which is also raised.

Sexes alike. Irides yellow, bill black, legs and feet brown. ♂ $9\frac{1}{2}$–$3\frac{3}{4}$–$3\frac{1}{4}$–$12\frac{1}{2}$; $9\frac{1}{4}$–$3\frac{3}{4}$–3–$12\frac{1}{4}$; 9–$3\frac{3}{4}$–$3\frac{1}{2}$–12; $9\frac{1}{2}$–$3\frac{3}{4}$–$3\frac{1}{2}$–12. ♀ 9–$3\frac{7}{8}$–$3\frac{1}{2}$.

"Nest closely resembles that of our Brown Thrush (*H. rufus*), but often built at a considerable distance from the ground. It is composed of fine twigs and grasses, generally with more or less mud. The eggs are said by the natives to be dark bluish green. All the nests found contained young. Breeds early, about the last of February."—(A. H. V.)

49. **Thryothorus rufescens** Lawr. "Rosingnole," Pat. (Fr. Rossignol, Nightingale). "Nightingale," Eng.

Plate xxvii, Fig. 1.

Rather common, but shy and difficult to procure. Found chiefly near the ground, generally in thickets and heavy undergrowth along

the borders of trails and openings. Its loud clear song is much oftener heard than the bird itself is seen, but by dint of quiet and patient waiting I have several times been able to observe its habits, which, together with its notes, resemble those of our House Wren (*Troglodytes aëdon*).

The male only, of this species, was described by Mr. Lawrence, as Mr. Ober did not take any females. We obtained two, and five males. The sexes do not differ but little, but the females are a shade lighter in color than the males, this being most noticeable on the throat and sides of the head and face. They also are slightly smaller than most of the males and the bill is very slightly shorter, about $\frac{1}{32}$ of an inch.

Two of the males vary from the rest; in one, taken March 8, at Laudat (the last one given in the list of measurements below), each feather of the under surface is lightly tipped with dark brown, strongest across the breast, wanting on the flanks. In the other specimen there is one white tertiary in the left wing, and on dissection the left testicle was found to be very rudimentary; whether there is any connection between the two facts I cannot say, but could find no traces of any injury.

Iris brown; upper mandible dark brown; the lower yellow, growing brown at the tip; legs and feet light brown. ♂ $5\frac{1}{4}$–2–$1\frac{5}{8}$–7; $5\frac{1}{4}$–2–$1\frac{1}{2}$–$6\frac{7}{8}$; 5–2–$1\frac{1}{4}$–$6\frac{1}{2}$; 5–$2\frac{1}{4}$–$1\frac{1}{2}$–$6\frac{1}{2}$; $4\frac{1}{2}$–2–$1\frac{1}{2}$. ♀ $4\frac{1}{2}$–$1\frac{3}{4}$–$1\frac{1}{2}$–$6\frac{1}{2}$; $4\frac{1}{2}$–2–$1\frac{11}{16}$. In all specimens, bill, from front, $\frac{9}{16}$, from rictus $\frac{3}{4}$, tarsus $\frac{11}{16}$.

"Nest built of sticks and roots in hollow logs, etc. Eggs generally two to six, very much like those of the 'Sucrier' (*C. dominicana*) but generally smaller. A nest with three eggs taken April 9th, 1890, at the Mountain Lake."—A. H. V.

The three eggs mentioned, measure ·74 × ·51, ·73 × ·51, ·59 × ·48, and do not differ appreciably in the color and markings from those of the "Sucrier."

## Family, TURDIDÆ.

50. **Myadestes dominicanus** Stejn. "Siffleur Montagne," Pat. and Fr. "Mountain Whistler," Eng.

Referred to *Myiadestes genibarbis* Sw. in LAWR. List.

Common but, from its shy and retiring habits, much more often heard than seen. Generally found in deep, dark woods, at an elevation of at least 800–1000 feet and for the most part higher. Its shy and solitary habits, more than two individuals being rarely seen together, are apparently due to natural disposition rather than

timidity, for it permits quite a close approach without manifesting fear or attempting to escape. In its movements it is, for the most part, slow and deliberate, very different from most of the thrushes. Its note is perhaps its most striking characteristic, and once heard, coming from the depths of some dark ravine, the author being usually entirely invisible, can never be forgotten. It is a loud and clear but rather melancholy whistle, slowly uttered and repeated at intervals, the bird usually remaining in the same place for some little time.

Sexes much alike. In most of our series of twelve specimens I can see no trace whatever of the olivaceous tint on the head, mentioned by Mr. Stejneger (Proc. U. S. Nat. Mus., vol. v, p. 22), and the same tint on the back does not seem to vary but very little with the sex, it being almost absent on some females, and quite plain on some males. Again, the olivaceous on the lores and over the eye seems to vary greatly, being decided in some, and almost absent in others, without regard to sex or season; so that I judge it, together with the tint on the back, to be more a matter of age or individual variation than a sexual or specific character, as the specimens that are strongest olivaceous on the back, have the most of the same tint on the lores and over the eye. In one, a female, taken May 15, where the tint on the back even approaches rufous and extends on to the wing coverts, the olivaceous on the head is very decided, the whole side of the head and some of the ear coverts being washed with it. Irides brown, legs and feet yellow, bill black ♂ $8\frac{5}{8}$–$3\frac{3}{4}$–$3\frac{7}{8}$–12 ; $8\frac{1}{2}$–$3\frac{1}{2}$–$3\frac{1}{2}$–$11\frac{1}{2}$ ; $8\frac{1}{4}$–$3\frac{3}{4}$–$3\frac{3}{4}$–$11\frac{1}{2}$. ♀ $8\frac{1}{4}$–$3\frac{1}{2}$–$3\frac{1}{2}$–$11\frac{3}{4}$ ; $8\frac{1}{4}$–$3\frac{5}{8}$–$3\frac{1}{2}$–$11\frac{5}{8}$ ; 8–$3\frac{5}{8}$–$3\frac{3}{4}$. One specimen, sex undetermined owing to mutilation, $7\frac{3}{4}$–$3\frac{3}{8}$–$3\frac{1}{2}$–$10\frac{1}{2}$.

"Nest a very frail structure of hair and roots, something like that of our Chipping Sparrow (*Spizella socialis*). Eggs, two in number, white, spotted with lilac about the larger end. One nest taken with badly incubated eggs which could not be saved. Situated about 8 feet from the ground in a lime tree. Laudat, April 9, 1890."—(A. H. V.)

51. **Allenia montana** (Lafr.). "Grive," "Grivette," Pat. and Fr. (Small Thrush).

*Margarops montanus* (Vieill.); Lawr., and Scl. List.

Common but, like the following, much hunted and hence rather shy. Widely distributed and found in much the same places as the next species, though as a rule nearer the ground, sometimes close to or on it. Song like that of the "Grosse Grive" but shorter.

Sexes alike. Irides of adult, white or pale yellow; of the young brown; bill black; legs and feet dark brown. ♂ 9½–4¾–3⅞–14½; 9–4⅜–3½. ♀ 9–4½–3½. ♀ juv. 9½–4½–3¼–12½.

"Nest like that of the 'Grosse Grive' (*C. fuscata densirostris*) but smaller."—(A. H. V.)

One set taken, May 3, at Spring Hill, contained very large embryos. These eggs are of a uniform blue green color and measure 1·14 × ·78, 1·11 × ·80 and 1·23 × ·79.

52. **Cichlherminia fuscata densirostris** (Vieill.). "Grosse Grive," Pat. and Fr. (Large Thrush).

*Margarops densirostris* (Vieill.); Lawr. List.

Not common and very wild. This and the foregoing are much hunted for food, which probably accounts for their shyness. Well distributed and found mainly in the tops of trees and seen flying over at considerable height. The song of this and the preceding is loud and rather pleasing but short, somewhat like that of our Wood Thrush (*Turdus mustelinus*), and is mainly uttered late in the afternoon, a short time before sunset.

Sexes much alike, the male apparently slightly larger. Irides white; legs, feet, and upper mandible yellowish brown; lower mandible yellow at the base, dark at the tip. ♂ 12½–5½–4½, extent not taken. ♀ 11¾–5⅜–4½–17⅝.

"Nest built of twigs and lianas, closely resembling that of the Brown Thrush (*Harporhynchus rufus*). Eggs blue. Several nests found with young. Breeds in February March."—(A. H. V.)

53. **Cichlherminia dominicensis** (Lawr.). "Morvy," Pat. (Fr. Mauvis, Redwing).

Referred to *Margarops herminieri* (Lafr.) in Lawr. List. Afterwards described by him as *M. dominicensis* (P. U. S. N. M., vol. iii, p. 16).

Not common and very shy. Found chiefly near the ground in the thick woods, Laudat, Bass-en-ville.

Note, a loud whistle somewhat resembling some of the notes of the Blue Jay (*Cyanocitta cristata*).

Sexes much alike, the female appears to have rather more white on the abdomen. Irides white; legs, feet and bare skin surrounding eyes, yellow; upper mandible yellowish brown, darker at the base; lower mandible yellow. ♂ 10⅛–4¾–3⅝–15⅝; 10¼–5–3½–16. ♀ 11–5–3¼; 11–5–3½; 11–4⅞–3.

One nest taken at Bass-en-ville, May 19th, contained only a few fragments of the eggs, of a beautiful, uniform blue green about the color of those of the "Grive" (*A. montana*). It was in thick,

swampy woods situated in the crotch of a small tree about ten feet from the ground and is a very handsome structure, composed mainly of long, dark green moss thickly lined with small roots and stems of plants. Diameter on top 5¼ in., on bottom 8 in., height 4 in. The cavity measures 3¾ in. across the top and 2½ in. deep. Plate xxv, fig. 4.

54. **Mimocichla verrillorum** Allen. "Perro-vanter," Pat.

PLATE XXVII, FIG. 3.

*M. ardesiaca* (Vieill.); Scl. List.

*M. ardesiaca albiventris*; Scl. List. in notes (P. Z. S. 1889, p. 326).

*M. albiventris* (Scl.); Allen in Auk, vol. viii, No. 3, p. 217.

Very rare and shy. The only two specimens obtained, a pair, were taken at Lasswa, on the windward or eastern side of the island, and apparently, from the testimony of the natives and our own experience, its habitat is mainly confined to that portion of the island, though once or twice its notes were heard near Bass-en-ville, and, as mentioned later on, it was taken on the western side the previous year, by Mr. Ramage.

"Note a shrill, plaintive whistle."—(A. H. V.)

Sexes much alike, but the female "slightly smaller than the male, with the breast paler and the abdomen more deeply tinted with yellowish buff."—(Allen.) Bill, legs and feet yellow; iris dark brown. Measurements, from skin: ♂ length, 10·50; wing, 4·60; tail, 4·50; culmen, 0·85.

Mr. J. A. Allen described this bird under the above name in the "Auk" for April, 1891 (vol. viii., No. 2, p. 217), considering it a new species, but later, in the next number of the same journal (p. 317), refers it to *M. albiventris* (Scl.), adopting as specific the quasi sub-specific name used by Dr. P. L. Sclater in his "List of Birds Collected by Mr. Ramage in Dominica, West Indies" (P. Z. S., 1889, p. 326), in speaking of two male specimens collected by Mr. Geo. A. Ramage at Batalie, on the leeward side of Dominica, in March, 1889.

In the enumerated list of Dominican birds at the head of his article, Dr. Sclater mentions the bird in question as *M. ardesiaca* (Vieill.) from which, as Mr. Allen has shown in his first article, it is quite distinct, but later on in the article he says, "as might have been expected the Dominican *Mimocichla* belongs to the Porto Rican form. It is, in fact, so nearly similiar that I do not see sufficient grounds for making it specifically distinct. The only difference apparent is the much greater whiteness of the belly in the

Dominican specimens whence, those who adopt trinomials, would, no doubt, call it *M. ardesiaca albiventris.*" And again, a little further on, he alludes to it as *M. ardesiaca albiventris* but gives no further description, and failed, as Mr. Allen remarks in his second paper, to point out some of the principal differences between this and *M. ardesiaca* (Vieill.) From this and the fact that he calls it *M. ardesiaca* (Vieill.) in his enumerated list at the head of his article it hardly seems as though he intended to recognize it as a sub-species, and certainly not as a distinct species, which it is clearly shown to be by the following characters given by Mr. Allen in his first article. "Similiar to *M. ardosciacea* of San Domingo and Porto Rico, but much smaller, with much more white on the tail, and with the abdomen strong buff instead of plumbeous fading into white." * * * "This species finds its nearest relative in *Mimocichla ardosciacea* of Porto Rico and Santo Domingo holding somewhat the same relation to it as regards the color of the ventral surface that *M. rubripes* holds to *M. plumbea.* The wing and tail are each fully three-fourths of an inch shorter in *M. verrillorum* than in *M. ardosciacea;* the culmen is also shorter; but the tarsi are slightly longer and the wing appreciably more rounded. The white in the tail is much purer and twice greater in extent, tipping the outer five pairs of feathers instead of being confined to the outer four, as in the other species of the genus, and occupying considerably more than the apical half of the outer feathers."

Not taken by Mr. Ober, but probably the "5. Thrush?" mentioned by Mr. Lawrence (Proc. U. S. Nat. Mus. i., 1878, p. 53), as having been "described by several persons, something like the Thrush, but with yellow bill and legs." Nest and eggs not taken but said by the natives to resemble those of the "Trembleur" (*C. ruficauda.*)

Appended is a list, complete so far as I have been able to ascertain, of all the birds that have been recorded from Dominica. It contains all the species recorded by Mr. E. C. Taylor, who was there in 1863; Mr. Ober, who was there in 1877; Mr. Ramage, who was there in 1887 and 1888; and my brother and myself, who were there in 1890, and shows what species were recorded by each collector, and whether actually obtained or observed by him.

It is, I believe, the most complete list, so far published, of the birds of this island, but it is not yet complete, probably by quite a number of species, and it is, I think, quite probable that in some of the denser and more unexplored parts of the island there may yet be found birds entirely unexpected there, or even undescribed species.

| | List of Birds recorded from Dominica. | By whom recorded. E. C. Taylor. | F. A. Ober. | G. A. Ramage. | G. E. and A. H. Verrill. |
|---|---|---|---|---|---|
| 1 | Sterna dougalli Montag. | -- | + | -- | -- |
| 2 | Sterna antillarum (Less.) | -- | + | -- | -- |
| 3 | Sterna fuliginosa Gmel. | -- | +1 | -- | ? |
| 4 | Sterna anæthetus Scop. | -- | + | -- | -- |
| 5 | Anous stolidus (Linn.) | -- | ? | -- | -- |
| 6 | Phaëthon flavirostris Brandt. | -- | + | -- | × |
| 7 | Pelecanus fuscus Linn | × | × | -- | × |
| 8 | Fregata aquila (Linn.) | × | × | -- | × |
| 9 | Ardea herodias Linn. | -- | -- | -- | × |
| 10 | Ardea egretta Gmel. | -- | -- | -- | × |
| 11 | Ardea candidissima Gmel. | -- | + | -- | (?) |
| 12 | Ardea cærulea Linn. | -- | +1 | -- | (?) |
| 13 | Ardea virescens Linn. | + | + | + | + |
| 14 | Nycticorax violaceus (Linn.) | -- | -- | + | + |
| 15 | Ionornis martinica (Linn.) | -- | +1 | -- | (?) |
| 16 | Ereunetes pusillus (Linn.) | -- | ? | -- | ? |
| 17 | Actitis macularia (Linn.) | -- | + | -- | × |
| 18 | Charadrius dominicus Müll. | -- | × | -- | -- |
| 19 | Arenaria interpres (Linn.) | -- | +1 | -- | -- |
| 20 | Columba corensis Gmel. | +1 | + | -- | + |
| 21 | Columba leucocephala Linn. | -- | -- | -- | × |
| 22 | Zenaida martinicana Bonap. | + | + | + | + |
| 23 | Columbigallina passerina (Linn.) | -- | + | + | + |
| 24 | Geotrygon montana (Linn.) | -- | + | -- | + |
| 25 | Geotrygon mystacea (Temm.) | -- | -- | -- | × |
| 26 | Buteo latissimus (Wils.) | -- | + | + | + |
| 27 | Falco columbarius Linn. | -- | -- | -- | + |
| 28 | Falco caribbæarum Gmel. | × | + | + | + ♂ |
| 29 | Pandion haliaëtus carolinensis (Gmel.) | -- | × | -- | × |
| 30 | Strix flammea nigrescens Lawr. | -- | + | -- | + ♂ |
| 31 | Amazona augusta (Vig.) | -- | + | + | + |
| 32 | Amazona bouqueti (Wagl.) | -- | ? | + | + ♂ |
| 33 | Coccyzus minor (Gmel.) | + | + | + | + |
| 34 | Ceryle alcyon (Linn.) | -- | × | -- | × |
| 35 | Cypseloides niger (Gmel.) | -- | ? | -- | + |
| 36 | Chætura dominicana Lawr. | -- | + | -- | + |
| 37 | Eulampis jugularis (Linn.) | + | + | + | + |
| 38 | Eulampis holosericeus (Linn.) | + | + | + | + |
| 39 | Thalurania bicolor (Gmel.) | -- | + | + | + |
| 40 | Bellona exilis (Gmel.) | + | + | + | + |
| 41 | Tyrannus rostratus Scl. | -- | + | + | + |
| 42 | Myiarchus tyrannulus oberi (Lawr.) | + | + | + | + |
| 43 | Blacicus brunneicapillus Lawr. | -- | + | + | + |
| 44 | Elænea pagana martinica (Linn.) | + | + | + | + |
| 45 | Loxigilla noctis sclateri Allen. | + | + | + | + |
| 46 | Euetheia bicolor (Linn.) | + | + | + | + |
| 47 | Euphonia flavifrons (Sparrm.) | -- | + ♀ | -- | + |
| 48 | Saltator guadelupensis Lafr. | + | + | + | + |
| 49 | Progne dominicensis (Gmel.) | -- | + | -- | ? |
| 50 | Vireo calidris (Linn.) | + | + | + | + |
| 51 | Cœreba dominicana (Taylor) | + | + | + | + |
| 52 | Dendroica petechia melanoptera Lawr. | + | + | + | + |

| | List of Birds recorded from Dominica. | By whom recorded. | | | |
|---|---|---|---|---|---|
| | | E. C. Taylor. | F. A. Ober. | G. A. Ramage. | G. E. and A. H. Verrill. |
| 53 | Dendroica plumbea Lawr. | -- | + | + | + |
| 54 | Dendroica virens (Gmel.) | -- | +1 | -- | -- |
| 55 | Seiurus noveboracensis (Gmel.) | -- | + | -- | (?) |
| 56 | Setophaga ruticilla (Linn.) | -- | + | + | + |
| 57 | Cinclocerthia ruficauda (Gld.) | -- | + | + | + |
| 58 | Thryothorus rufescens Lawr. | -- | + ♂ | + | + |
| 59 | Myadestes dominicanus Stejn. | -- | + | + | + |
| 60 | Allenia montana (Lafr.) | -- | + | + | + |
| 61 | Cichlherminia fuscata densirostris (Vieill.) | -- | + | -- | + |
| 62 | Cichlherminia dominicensis (Lawr.) | -- | + ♂ | -- | + |
| 63 | Mimocichla verrillorum Allen | -- | (?) | + ♂ | + |

The following signs are used in the above table: + = actually obtained; × = observed and species identified but not obtained; ?= observed and probably of that species but not positively identified; (?)= described by the inhabitants and probably of that species but not seen by the collectors themselves; + ♂ or + ♀ indicates that the sex indicated only was obtained. In several cases only one specimen was taken by the former collectors but the sex is not mentioned by them in their lists, in such cases it is indicated thus, + 1.

To this list should be added *Tringa minutilla* Vieill. taken in Dominica by Dr. Nicholls, and sent to the Smithsonian in 1880. He also sent ten other species, all of them included in the above list; but the three following, though they were observed, have not been actually taken there by any of the above collectors.

*Anous stolidus.*
*Ereunetes pusillus.*
*Charadrius dominicus.*

## *List of Batrachians and Reptiles obtained.*

## BATRACHIA.

### Order, ANURA.

#### Family, Cystignathidæ.

1. **Leptodactylus pentadactylus** Lawr. "Crapaud," Pat. and Fr. (Toad.)

Common and much used for food. When used for the table the whole animal is eaten, generally as a stew, and not simply the legs, as is the case with frogs in this country. Prepared in this way it makes a delicious dish, tasting much like chicken, but more delicate.

2. **Hylodes martinicensis** D. and B. Tree Toad.

Rather common. All our specimens were taken at a considerable elevation (1000–2000 feet), and were found under stones or logs.

## REPTILIA.

### Order, OPHIDIA.

#### Family, TYPHLOPIDÆ.

3. **Typhlops lumbricalis** Linn. "Blind Worm."

Apparently rare. We only obtained one specimen and no others were seen.

#### Family, BOIDÆ.

4. **Boa diviniloquax** Daud. "Boa."

Rather common. Our specimens were all obtained in the interior, near Bass-en-ville, and apparently it is found mainly in the densely wooded and elevated parts of the island. This species sometimes attains large size. We heard of their being taken 12 or 13 feet long, but none of our specimens were over 7 or 8 feet in length. Three were brought to New Haven alive.

#### Family, COLUBRIDÆ.

5. **Opheomorphus juliae** Cope. "Snake."

Not particularly common. This, the preceding species, and the "Blind Worm" were the only snakes found by us on the island, though Mr. Ober took a fourth, *Alsophis sibonius* Cope. This species varies much in color and general appearance so that the natives, and we ourselves, thought there were several species among them. In one specimen, the largest, 21 inches long, the round yellow spots near the base of the scales, spoken of by Mr. Cope, are entirely lacking.

### Order, LACERTILIA.

#### Family, GECKONIDÆ.

6. **Sphærodactylus oxyrhinus** Gosse. Gecko.

One specimen only obtained, at Bass-en-ville, and no others were observed.

#### Family, IGUANIDÆ.

7. **Iguana delicatissima** Lawr. "Iguana."

Rather common. Frequently used as food. My brother states that the flesh is very fair eating.

8. **Anolis leachii** Gray. "Chameleon."

Very abundant and widely distributed. Found at all elevations. A very lively and beautiful species. A peculiarity of this lizard is the effect upon it of whistling or music, causing it to stop and listen attentively and even allow itself to be caught.

### Family, Teiidæ.

9. **Amiva plei** D. and B., var. **brachiosquamatus**, nov. Cope. "Arbalo" (Pat.).

Prof. E. D. Cope describes this new sub-species as follows: "Differs from typical forms in having numerous rows of small brachial scales as in *A. major*, instead of one large row as in typical *A. plei*. Three supraorbital plates. Otherwise as in *A. plei*."

Very abundant in the lowlands, particularly in the cane-fields, where on a hot, sunny day they may be seen by hundreds. They are exceedingly quick in their motions and run with great rapidity, so that they can only be procured by shooting. This species grows very large, frequently attaining a length of two or three feet.

I think there are at least two species of "Arbalos," but we only succeeded in obtaining one.

### Family, Scincidæ.

10. **Mabuia agilis nigropunctata** Spix. Skink.

Common, but shy and difficult to catch. Generally found in rather damp places and at quite an elevation.

## Order, CHELONIA.

### Family, Testudinidæ.

11. **Testudo tabulata** Linn. Turtle.

Our specimen came from the island of Tortola, but we were told that they were also found in Dominica, though rare.

*Species obtained by Mr. Fred. A. Ober, in addition to the above List.*

Mr. Ober obtained four species of reptiles (no batrachians), in Dominica, which are described by Prof. E. D. Cope in the Proc. Amer. Philos. Soc., vol. xviii, p. 274.

Of these four species one was also obtained by us, *Opheomorphus juliæ*, the others I give below to complete so far as possible, the herpetology of this island, though I feel sure the list of species is still far from complete, especially among the lizards.

12. (1.) **Mabuia cepedei** Gray.

13. (2.) **Xiphosurus oculatus** Cope.

14. (3.) **Alsophis sibonius** Cope.

*List of Land and Fresh-water Crustacea obtained.*

1. **Palæmon jamaicensis** Oliv. "Crawfish." Prawn.

Common in the fresh-water streams and extensively used as food, being of excellent flavor.

One or more species of small grey shrimp were frequently seen in the river at Bass-en-ville, but owing to their very lively habits and our lack of proper means we were unable to procure any.

2. **Cenobita diogenes** Latr. "Sojer" (Soldier). Hermit Crab.

Not very common.

"Found only on the windward or Atlantic side of the island."—(A. H. V.)

3. **Gecarcinus ruricola** Latr. "Crabe," Pat. and Fr. (Crab). Land Crab.

Common, but not found among the mountains nor at any great elevation. Very good eating and much used for food.

4. **Pseudotelphusa dentata** (H. Milne-Edw.). "Surique," Pat. Land Crab.

*Pseudotelphusa tenuipes.* R. I. Pocock (Ann. and Mag. Nat. Hist., vol. iii, p. 7, 1889).

Very common in the interior and among the mountains. We found this species extremely abundant in the neighborhood of Bass-en-ville. They were seen running around everywhere in the woods, though also found along the shores of the streams and in the streams themselves, but apparently it made no difference whether water was near or not, though of course the ground is everywhere very damp from the frequent rains. When disturbed or pursued they run very rapidly and generally get into some hole or under a log or stone, but I could never see that they had any particular hole or burrow. When unable to reach some such shelter they sit back on the hind part of the carapax, after the manner of our common Fiddler Crab, and defend themselves savagely. By throwing out a piece of meat a number could always be brought around the camp in a very short time.

Though not usually sold in the market, like the former species, and not considered so good eating, they are by no means bad, as I can testify from personal experience, and they are frequently eaten by the natives up among the mountains.

Mr. R. I. Pocock in the Ann. and Mag., vol. iii, p. 6, 1889, has recorded an additional species of land crab (*G. lateralis*), and several additional species of shrimp and prawns collected in Dominica by Mr. Ramage.

*Notes on the Insects.*

The collection of insects has not yet been worked up and so no list of the species can be given.

We found the Coleoptera and Lepidoptera quite abundant, the other orders much less so.

Our collection contains about twenty species of beetles, some of which are very interesting, to an ordinary observer at least, on account of their difference from New England forms.

Probably the most striking is the Hercules Beetle. These enormous beetles are quite rare and found mainly among the mountains. According to the inhabitants there are several species, but we procured but one, dark brown with greenish grey wing coverts, spotted with black. According to my brother, they feed on the locust and cocobolo trees, and he also says that on the windward side of the island this species is replaced by another that is still larger and entirely brown.

Another large brown "horn-bug" has a big white larva, called in Patois "Gru Gru Worm" which lives in the buds of the palm trees, and which is roasted and eaten by the natives.

The "fire-beetles" of this island are of several species, very numerous, and exceedingly brilliant, the light being constant and coming from the body under the wing coverts and from two round spots on the side of the thorax. They are a kind of snapping-beetle, dark brown, and about an inch long. A few of them flying about a room make enough light to distinguish large objects, and two or three in a small bottle give light enough, when held near the page, to read ordinary print. They are found mainly in the interior. At Bassen-ville they were very abundant.

We also took a number of large yellow and black weevils which were found on the plantain and banana plants.

The name "La Belle" is applied by the natives to all beetles.

Several large green katydids, called by the inhabitants "Crak Crak," were also taken, and enormous cockroaches, or "Drummers," were very abundant, especially in the houses.

We also found a few mole-crickets and a walking-stick but they were both rare.

No true scorpions were seen but a large whip-scorpion was obtained and also several large myriapods or centipedes.

The collection of Land Mollusca, consisting of about twenty species, several of them probably new, is now in the hands of Mr. H. A. Pilsbry of Philadelphia, for working up. As soon as this is done a list will be published.

## EXPLANATION OF PLATES.

### PLATE XXV.

Fig. 1. Nest and female of *Eulampis jugularis*, "Fou Fou Mardet," ½ natural size. p. 331.

Fig. 2. Cup-shaped nest and eggs of *Eulampis holosericeus*, "Fou Fou Tête-longue," ½ natural size. p. 332.

Fig. 3. Nest of *Cœreba dominicana*, "Sucrier," ⅔ natural size. p. 341.

Fig. 4. Nest of *Cichlherminia dominicensis*, "Morvy," $\frac{8}{10}$ natural size. p. 347.

Figs. 5 and 6. Eggs of *Blacicus brunneicapillus*, "Gobe-mouche," natural size. p. 337.

Figs. 7 and 8. Eggs of *Elænea payana martinica*, "Cheweck," natural size. p. 337.

Figs. 9 and 10. Eggs of *Cœreba dominicana*, "Sucrier," natural size. p. 341.

Fig. 11. Egg of *Dendroica petechia melanoptera*, "Teeteen," natural size. p. 342.

### PLATE XXVI.

Figs. 1 and 2. Nests of *Eulampis holosericeus*, "Fou Fou Tête-longue," natural size. p. 332.

Fig. 3. Nest of *Bellona exilis*, "Fou Fou Bequar," natural size. p. 335.

Figs. 4 and 5. Nests of *Thalurania bicolor*, "Fou Fou Bleu," natural size. pp. 334 and 333.

Fig. 6. Nest of *Bellona exilis* "Fou Fou Bequar," natural size. p. 334.

### PLATE XXVII.

Fig. 1. *Thryothorus rufescens*, "Rosingnole," adult male, ⅔ natural size. p. 343.

Fig. 2. *Dendroica plumbea*, "Pa-pia," adult female, ⅔ natural size. p. 342.

Fig. 3. *Mimocichla verrillorum*, "Perro-vanter," adult female, ½ natural size. p. 347.

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The above photo-lithographic plates, by Mr. E. Crisand, of New Haven, are made directly from photographs of the original specimens.

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NOTE.—Since the previous pages were printed I find that in preparing my list of the Reptiles and Batrachians, I unfortunately overlooked an article by Dr. A. Günther (Ann. and Mag. Nat. Hist. (6), II, p. 362), on the collection made by Mr. Ramage, and one by Mr. Samuel Garman (Bull. Ess. Inst., XIX, p. 1), on the collections in the Museum of Comparative Zoology, at Cambridge.

Dr. Günther enumerates ten species, of which two, *Thecadactylus rapicauda* (Houtt.) and *Ameiva fuscata* Garman (l. c. p 5), are additional to my list, though the latter is probably the other "Arbalo" seen, but not obtained, by us. *Sphærodactylus copii* (Steind.), also recorded by him, is possibly the same as our *S. oxyrhinus* Gosse. In this paper Dr. Günther unites *Xiphosurus oculatus* Cope with *Anolis alliaceus* Cope, and *Mabuia dominicana* Garman with *M. agilis* (Radde), of these, the two former are doubtless the same as our *A. leachii* Gray, and the two latter identical with our *M. agilis nigropunctata* Spix.

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ERRATUM.—Page 350, fifth line from bottom, and page 351, third line from bottom, for "Lawr." read "Laur."

## XX.—On a Collection of Land Mollusca from the Island of Dominica, West Indies. By Henry A. Pilsbry.

Collections of the land mollusks of Dominica have been made by Mr. R. Lechmere Guppy,* Mr. A. D. Brown,† Mr. G. F. Angas,‡ Mr. G. A. Ramage§ and Dr. Benj. Sharp.‖ The present list is based upon a collection made in March, April and May, 1890, by Messrs. G. E. and A. H. Verrill.¶

In Mr. E. A. Smith's reports upon the collections made by Mr. Ramage, a full list of the land molluscan fauna of the island is compiled from the reports of previous investigators. To that paper, therefore, the student is referred for references to the literature of the fauna.

### HELICIDÆ.

**Helix (Dentellaria) nigrescens** Wood. (11845).**

A color variety (11854) is yellowish-olive, becoming brown toward the lip and in front of the aperture.

**H. (Dentellaria) badia** Fér. (11826).

**H. (Dentellaria) josephinæ** Fér. (11844, 11829).

### BULIMULIDÆ.

**Bulimulus laticinctus** Guppy. (11846).

This is no doubt a local variety of the five-banded *B. multifasciatus*, in which two of the bands are confluent, the band formula being 1(23)45, whilst that of typical *multifasciatus* is 12345.

* See Guppy's report in Annals and Magazine of Nat. Hist. (4), I, p. 429, 1868.

† American Naturalist, 1881, p. 56. Mr. Brown's specimens are now in the collection of the Academy of Natural Sciences of Philadelphia.

‡ Proc. Zool. Soc., 1883, p. 594.

§ See E. A. Smith's reports in Ann. and Mag. N. H. (6) II, p. 227, 419.

‖ The specimens collected by Dr. Sharp are in the collection of the Academy of Natural Sciences of Philadelphia.

¶ This collection is in the Museum of Yale College.

** The figures refer to the museum numbers of the Yale College collection.

**B. exilis** Gmel. (11827, 11856, 11851, 11855, 11847).

The following color-forms are represented:

(1) Horn colored, bandless (11827).
(2) Horn colored, having a faint peripheral band (11847, 11851).
(3) Horn colored, having a conspicuous dark band (11856).
(4) Having three dark purple-brown bands, the upper and lower bands wider (11855).

**B. nichollsii** A. D. Brown. (11850, 11848, 11849).

A species peculiar to Dominica.

**Amphibulima patula** Brug. (11828, 11835).

## STENOGYRIDÆ.

**Stenogyra octona** Chemn. (11852, 11840).

## VAGINULIDÆ.

**Vaginula punctatissima** Semper. (11830).

Not before reported from Dominica. The black blotches of the ventral surface are much more conspicuous than one would suppose from Semper's figure.

## HELICINIDÆ.

**Helicina rhodostoma** Gray. (11842, 11853, 11841).

The usual variations in lip coloring, from yellow to deep red, are represented.

**H. epistilia** Guppy. (11839).

A single specimen may be referred to this unfigured and rare species.

**H. velutina** Guppy. (11837, 11838).

The specimens are a trifle more depressed than *H. rufa* Pfr. from Yuma, Haiti, but the difference is so slight that I am wholly disposed to consider Guppy's species a synonym of the earlier described Haitian form. Pfeiffer's description of *H. rufa* is imperfect, as the delicate *velvety* character of the cuticle is not noticed by him. I have, however, compared specimens from Yuma with the Dominican shells, and am satisfied that they are identical.

## NERITIDÆ.

**Neritina punctulata** Lam. (11825).

The specimens were collected from the Layou River at Bass-en-Ville, at an altitude of about 2000 ft. They are large, and have the mouth-callus of a dull reddish color.

## CYCLOPHORIDÆ.

**Cyclophorus (Amphicyclotus) amethystinus** Guppy. (11843).

There can be no doubt that this species is distinct from *C. schrammi*, although the young greatly resemble the latter. It is known only from Dominica.

NOTE. — In the above article it will be seen that Mr. Pilsbry makes but fourteen species and none of them are new, several specimens which had been supposed, in a hasty examination, to be different species proving to be only color varieties of the other described forms.

The small number of species obtained by us was doubtless due to the fact that our collections, as stated at the beginning of my article, were made almost exclusively at a considerable elevation (from 1000 to 2500 feet), and no doubt mainly above the range of many species such as *Succinea approximans*, *Amphibulima rubescens*, *Leptinaria lamellata*, *Helix dentiens*, *Helicina fasciata*, *H. antillarum*, etc.

A large part of the Mollusca were collected in the vicinity of Bass-en-ville, at about 2000 feet elevation, and in the midst of the deep woods, which may also account for the absence of some species that frequent the gardens and plantations.

Here we found *Helicina rhodostoma* abundant on the trunks of trees and old stumps about our camp.

The Layou River flowed past our camp at this point, and in it *Neritina punctulata* was exceedingly abundant. All the specimens we saw, and we examined hundreds, were marked by cavities, varying much in size but often very deep and wide, which had the appearance of having been drilled or bored out. In many cases these holes covered the whole shell, but I think we never found any that had penetrated entirely through it.

*Stenogyra octona* was very abundant under logs and stones in the vicinity of all our camps.

*Vaginula punctatissima* was not common, but we found several specimens under logs and stones in the vicinity of Spring Hill.

*Bulimulus laticinctus* was found rather commonly near Laudat on the plantain and banana plants.

In Mr. E. A. Smith's articles, mentioned above by Mr. Pilsbry, there are enumerated thirty-four species of land mollusks inhabiting Dominica; of these a few seem to be of doubtful identity, or hardly entitled to specific rank, but there are probably over thirty good species now known from this island.—G. E. VERRILL.

# XXI.—New England Spiders of the Family Thomisidæ.

By J. H. Emerton.

## Thomisidæ.

In the *Thomisidæ* the cephalothorax and abdomen are both flattened. The cephalothorax is about as wide as long, and the abdomen is usually widest behind. The legs are turned outward and forward more than downward, so that the body is carried close to the ground, and some species walk more readily sidewise than forward.

The family consists of two groups or sub-families, the *Thomisinæ* and the *Philodrominæ*.

In the *Thomisinæ* the first and second pairs of legs are about equal in length and much longer than the third and fourth. The feet have two claws and under them a small cluster of hairs like the rest of the hairs on the leg.

In the *Philodrominæ* the legs are more nearly equal in length, all long and slender, and the second pair usually longest. The feet have two claws and under them a large cluster of long hairs, widened and flat at the end.

The eyes of the *Thomisidæ* are small and nearly of the same size and have a simple arrangement in two rows. The maxillæ are short and narrow at the ends. The males usually differ considerably in size and color from the females, and in some species the males are very much smaller than the females.

The *Thomisidæ* live mostly on plants, and in winter hide in cracks and under stones and bark. Their colors resemble the plants on which they live, most species being marked with gray and brown like bark, while those living on flowers are brightly colored, white and yellow. They make no webs and no nests except a few threads to hold the cocoon and conceal it by drawing together a leaf over it.

A large part of the New England species have been described, a few of them by Hentz in the Journal of the Boston Soc. Nat. Hist., vol. v, but more by E. Keyserling in the Proceedings of the zool. botan. gesellschaft of Vienna and in a separate book on the "Spinnen Americas, Laterigradæ," published in 1880. The spiders in the Museum of Comparative Zoology at Cambridge have been named by Keyserling and among them are several of the New England species which have been compared with my own specimens.

While this paper was printing, Mr. Nathan Banks published in the Proc. Acad. Nat. Sci of Philadelphia, a Catalogue of the spiders

of the upper Cayuga Lake basin (Ithaca, N. Y.), with descriptions of many new species. Mr. Banks has let me examine his spiders and his names have been used for several species.

## Xysticus.

The body is short and flat. The cephalothorax is as wide as long, nearly square in front and half as wide across the eyes as it is at the widest part.

The front row of eyes is almost straight and the four middle eyes form a square or a rectangle wider than long.

The abdomen is not much larger than the cephalothorax, widest across the hinder half and not pointed behind. The legs are short in the females, the first and second pairs nearly equal and longer than the third and fourth which are also of nearly the same length. The males have the legs longer and the first and second pairs proportionally longer than the females.

The males are a little smaller than the females and darker colored. The male palpi are short. The tibia has a short simple process on the outer side and a crooked and more complicated one underneath. The tube of the palpal organ begins on the inner side of the tarsus and extends around the bulb to the outer side where it rests in a small thin process turned outward. On the under side of the bulb are two processes of various shapes in different species.

The epigynum has a rounded opening variable in shape even in the same species, sometimes simple and sometimes divided by a median ridge.

**Xysticus limbatus** Keys., Spinnen Americas, 1880.

The female *X. crudelis* Banks, and *X. brunneus* Banks, appear to be this species, but the male *X. limbatus* Banks is different from my *X. limbatus* and nearer the male *X. limbatus* Keyserling.

PLATE XXVIII, FIGURES 1–1*h*.

This is one of the largest species, the females measuring 8 or $10^{mm}$ long and the cephalothorax $4^{mm}$ wide.

The colors are light brown spots of various shades on a nearly white ground, some individuals being very pale and others nearly covered by the brown spots. In half grown individuals the markings are most distinct and are like those of Hentz's figure of *Thomisus ferox* which is very likely this species, Pl. XXVIII, fig. 1*b*. In these the sides of the thorax are light brown and the abdomen has on each side three or four nearly square brown spots darkest on the

front and outer edges. The middle of the cephalothorax has a light marking tapering behind to the dorsal groove which is marked by a dark spot. The legs have a distinct fine light line on the dorsal side and are marked all over with small light brown spots without any distinct bands or patches except that the fourth femur and tibia are darker at the ends. In the adults the markings are less distinct and that of the middle of the cephalothorax darker so as to obscure the lateral stripes. Pl. XXVIII, figs. 1, 1*a*. The males have the same markings but darker. Fig. 1*a*. The epigynum, figs. 1*d*, 1*e*, 1*f*, has a simple opening of various shapes, sometimes with a low and indistinct ridge through the middle. The male palpus has a large and complicated hook under the tibia turned a little toward the outer side so as to be visible from above. Fig. 1*h*. The processes of the bulb are large and dark colored, the under one pointed and directed inward, and the other wide at the end and turned backward to the base of the bulb. Fig. 1*g*.

Males from Medford and Peabody, Mass. Females, Dedham, Peabody, Cambridge, Malden, Salem, Mass; Simsbury, Conn. Both sexes from Brooklyn, Long Island, N. Y., N. Pike. A female with cocoon partly covered by a folded leaf was found June 10.

The specimens in the Museum Comp. Zool. at Cambridge, named by Keyserling, are all females. The male which I suppose to belong to this species is not the *limbatus* Keys., but probably *X. elegans* Keys.

### Xysticus gulosus Keys.

♂ = *X. locuples* Keys., Spinnen Americas, 1880; and *X. lentus* Banks.

PLATE XXVIII, FIGURES 2–2*c*.

The female is 6 to 8$^{mm}$ long with the cephalothorax 3$^{mm}$ wide. The color is grayish brown and very uniform, the usual marking showing indistinctly among the fine brown spots that nearly cover the body. The femora are light on the ventral side and have two or three large spots along the middle. The femora are darker toward the end, those of the fourth pair having a distinct black spot near the end. The abdomen has usually only a pair of irregular black lines across near the middle, and several smaller and less distinct behind it. Pl. XXVIII, figs. 2, 2*a*.

The epigynum has an oval opening wider than long, in which are two oval ridges directed backward. Fig. 2*c*.

The male palpus has the under tibial process wide and turned outward at the end. The tube of the palpal organ is unusually long and

slender and has a long outward curve at the tip and a long thin process to support it. Fig. 2*b*.

Salem and Beverly, Mass., under stones; Long Island, N. Y., N. Pike.

### Xysticus stomachosus Keys., Spinnen Americas, 1880.

A small female apparently of this species is named *X. benefactor* in the Mus. Comp. Zool. Cambridge.

PLATE XXVIII, FIGURES 3–3*d*.

This is a little smaller than *limbatus*. A mature female measures $6^{mm}$ long and the thorax $3^{mm}$ wide. The largest males are as large as small males of *limbatus*. The brown markings are grayer than in *limbatus* and less evenly distributed over the body. The lateral dark bands of the cephalothorax are very distinct and have a darker line on the outer edge and two darker spots behind under the front of the abdomen. The middle of the cephalothorax is light, but a little darker toward the front of the head.

The brown markings of the abdomen are light with black spots along the front and outer edges of each marking. The first and second legs are covered with small brown spots darker and closer at the ends of the joints. The third and fourth legs are lighter and have darker spots at the ends of the joints, the fourth legs having conspicuous dark spots on the ends of patella and tibia. Pl. XXVIII, figs. 3, 3*a*.

The epigynum has a wider opening than *limbatus*, divided by a flat ridge at the hinder edge. Fig. 3*d*.

The male palpus has the under process of the tibia small and divided at the end into two blunt knobs. The tube is stout and black and ends between two large thin processes on the outer side. Pl. XXVIII, figs. 3*b*, 3*c*. The processes of the bulb are small but extend outward from its surface. The anterior process is attached near its middle and pointed at both ends. Figs. 3*b*, 3*c*.

Readville, Brookline, Saugus, Swampscott, Mass.; New Haven, Conn.; Long Island, N. Y., N. Pike's collection.

### Xysticus nervosus Banks.

PLATE XXVIII, FIGURES 4–4*d*.

Female $6^{mm}$ long, and cephalothorax $3^{mm}$ wide. The markings of both sexes are very indistinct and much alike. The general color is light brownish yellow with darker and lighter markings scattered in

small spots all over the body. The middle of the cephalothorax is as usual light colored with a few brown markings in the middle and toward the eyes. The sides of the cephalothorax are darker with irregular brownish lines. The abdomen has three or four pairs of indistinct brown spots, in front of which and on the front of the abdomen are irregular small white spots. The legs are marked in the same way with irregular light and dark spots, the first and second pairs a little darker than the others. Pl. XXVIII, figs. 4, 4*a*.

In the males the first and second legs are very long and slender, the second twice as long as the third pair. The markings are a little brighter and more distinct than in the female. The under process of the tibia of the male palpus is curved and turned outward at the end as in *gulosus*. The processes of the bulb are long and the anterior one has a complicated twist at the end. Figs. 4*c*, 4*d*, 4*e*.

The epigynum has a large oval opening, widest behind, in which are two smaller openings divided by a narrow ridge, fig. 4*b*.

Medford, Readville, Mass.; Long Island, N. Y., N. Pike.

**Xysticus triguttatus** Keys., Spinnen Americas, 1880.

The spider named *X. feroclus* by Keyserling in the Museum of Comp. Zoology, Cambridge, is the female of this species and so is *X. feroclus* Banks.

PLATE XXIX, FIGURES 1–1*d*.

The female is 5 or 6$^{mm}$ long and the cephalothorax 2$^{mm}$. The legs of the female are white or yellowish with a few black hairs and small spots around the ends of the femora. The cephalothorax is yellowish brown with a line of black marks each side ending in a distinct black spot at the hinder end. The third black spot is just behind the dorsal groove. The middle of the cephalothorax is very light behind and darker toward the front and middle of the head, Pl. XXIX, fig. 1, and the area between the eyes is white. The abdomen is white with two small black spots in front and several lines of black spots broken in the middle across the hinder half.

The male is as large as the female and much darker in color. The markings of the cephalothorax are like those of the female, but darker. The femora of the first and second legs are dark brown and the rest of the legs dull yellow. The tarsus of the male palpus is white and the rest dark. The abdomen has very dark markings, those of the hinder half usually running entirely across, with white between. Fig. 1*a*.

The epigynum has a large shallow oval opening, in the middle of which is a small hole with a hard projecting ridge each side. Fig. 1*c*.

The male palpus has the tube stout and extending almost entirely around it. The processes of the bulb are small and the posterior one blunt and notched at the end. Fig. 1*d*.

This is a very common species in grass and low plants all over New England. In Psyche, vol. v, I have described the pairing of this species. The female held herself on a blade of grass, head downward, with the abdomen turned away far enough for the male to reach under, from above, to the epigynum.

**Xysticus graminis**, new sp.

PLATE XXIX, FIGURES 2–2*b*.

This species resembles in color and markings the male of *X. triguttatus*. It is a little larger and darker, and there is less contrast between the light and dark portions. The legs are shorter and stouter and the patella and tibia of the first and second legs, as well as the femur, are dark brown. The ends of these legs, as well as the third and fourth pairs, are light yellow brown, not as white as in *triguttatus*. The dark bands on the cephalothorax are not so distinctly broken into black marks as in *triguttatus*, nor are the three spots as distinct. The markings of the abdomen do not extend across the front half and the lighter parts are not as white as in *triguttatus*. Pl. XXIX, fig. 2.

The male palpus resembles that of *triguttatus*, but the processes of the bulb are longer and both pointed and directed inward. Fig. 2*a*.

The female is $6^{mm}$ long and the cephalothorax is $3^{mm}$ long and $3^{mm}$ wide. The legs are short and stout. The color is dark brown with very small light markings. The front of the head is wide and light colored, with a distinct white band under the upper row of eyes, behind which is a very dark brown line. The dark patches on the sides of the thorax are partly divided into two, the inner halves ending in darker spots, as in *triguttatus* and *stomachosus*, but less distinctly marked. In the middle of the cephalothorax is a triangular dark area narrowing to a dark spot behind, and each side of this a light line narrowing forward to the eyes. The abdomen is dark with alternate narrow darker and white markings across the hinder half and obliquely down the sides. The legs are covered with small brown spots. The first and second femora are lighter on the front side, and the third and fourth legs have a light line on the dorsal side. The femora of the third and fourth legs have a dark spot at the end. The under side is dark with small brown spots, and the

spinnerets and epigynum are each covered by a very dark round spot. The epigynum resembles that of *triguttatus.*

Males from Peabody and Saugus, Mass. Females, Brookline, Mass., S. Henshaw.

**Xysticus formosus** Banks.

PLATE XXIX, FIGURES 3, 3*a*.

The male is $5^{mm}$ long and the cephalothorax $2{\cdot}5^{mm}$ wide. The dark sides of the cephalothorax extend toward the middle behind the eyes so that the middle light portion is narrower than usual. The parts around the eyes are white and so is the back of the cephalothorax around the dorsal groove. The hinder ends of the dark bands are nearly black where they pass under the front part of the abdomen. The abdomen is white in the middle with irregular brown spots, and the sides are dark brown, in angular patches that do not extend as far toward the middle as usual. The legs are covered with irregular brown spots and the patella and tibia of the first and second legs are much darker than the rest. Pl. XXIX, fig. 2.

The tibia of the male palpus has a very small hook underneath, but the outer process is longer than usual and between it and the inferior process is a wide flat tooth. Fig. 2*a*. The palpal organ is very simple, the usual processes of the bulb being represented only by a very short flat ridge. Fig. 2*a*.

Only one male from West Roxbury, Mass.

**Xysticus quadrilineatus** Keys., Spinnen Americas, 1880.

PLATE XXIX, FIGURES 4, 4*a*.

Female $7^{mm}$ long. Cephalothorax $2{\cdot}5^{mm}$ wide. In this species the cephalothorax is unusually wide in front. The color is light yellow with light brown markings and black spots. The cephalothorax has four narrow brown stripes, one each side close to the edge, and the others running back from the lateral eyes. There are also two fine brown lines in the middle, sometimes extending from the eyes to the dorsal groove, but usually broken in the middle. There is a brown spot just behind the dorsal groove and two others on the middle of the back, half way between the groove and the middle eyes. On the abdomen there are two black spots at the front end, two in the middle and two near the hinder end, besides several smaller ones along the sides. There are four light brown lines across the hinder half, each with a white line behind it, and there are oblique brown lines alternating with white at the sides. Pl. XXIX, fig. 4. The

legs have the usual light line along the dorsal side and are covered with fine brown spots without any distinct markings. The epigynum has an oblong opening, widest behind. Fig. 4*a*.

Medford, Swampscott, Beverly, Mass.; Long Island, N. Y., N. Pike.

### Xysticus inornatus, new sp.

PLATE XXIX, FIGURES 5–5*b*.

The adult female is $5^{mm}$ long, with the cephalothorax $2^{mm}$ wide. It is less flattened than most species and has the cephalothorax rounded up in the middle, where it is much higher than the eyes. Pl. XXIX, fig. 5. The cephalothorax is dark brown, almost black, with a lighter line in the middle and a white line each side near the edge. The legs are colored in the same way, but the thin parts between the joints are white. The tarsi of all the legs are lighter than the rest. At the base of the first and second femora, in front, are white spots, and the third and fourth legs have white longitudinal stripes. The under side of the cephalothorax and legs are of the same color, with white joints. The abdomen is light gray with indistinct lighter lines at the sides and small light spots in the middle. Pl. XXIX, fig. 5. The epigynum has a small opening, with two wide anterior and two sharp posterior projections from its edges. Fig. 5*b*.

Two young specimens less than half as large, have all the dark portions light yellowish brown.

Adult from Medford, Mass.; young from Beverly, Mass., and New Haven, Conn.

## Oxyptila Simon, 1864.

This genus differs little from *Xysticus*. The cephalothorax is flatter in the middle and the head narrower. The middle eyes of both rows are nearer together so that they form a rectangle longer than wide. The middle eyes of the front row and usually of both rows are farther forward than in *Xysticus*, so that both rows of eyes are more curved. There is less difference between the length of the front and hind legs in the males than in *Xysticus*. The dark markings of the thorax often approach each other behind, as in our species.

### Oxyptila cinerea, new sp.

PLATE XXIX, FIGURES 6, 6*a*.

This male is $4{\cdot}5^{mm}$ long and the cephalothorax $2{\cdot}5^{mm}$ long and $2^{mm}$ wide. The colors are grayish brown and white. The light portion

of the cephalothorax is narrowed near the dorsal groove and widened in front a little behind the eyes. Pl. XXIX, fig. 6. The abdomen has the usual markings, all indistinct. The legs are marked more nearly alike than in the other species. The femora are all thickly spotted with brown, and the third and fourth have a large dark spot at the end. The other joints are all light with dark rings at the ends. The inferior process of the tibia of the male palpus is large, rounded at the base, and turned outward at the tip. Pl. XXIX, fig. 6*a*. The processes of the bulb are small and both turned inward at the points. The tarsus is curved downward a little at the end.

One male, only, from the White Mountains, New Hampshire.

### Coriarachne Thorell.

The cephalothorax and abdomen are both very much flattened, not more than half as high in proportion to their width as in *Xysticus*. The cephalothorax is flat on the top, not rounded up from the middle, as in *Xysticus*. The head is narrower and more distinctly separated from the thorax by depressions at the sides. The arrangement of the eyes is the same as in *Xysticus*, except that the two rows are nearer together and lower. The male palpus has a small thin tooth on the end of the outer process of the tibia and there are no appendages to the under side of the bulb as there are in *Xysticus*.

**Coriarachne versicolor** Keys., Spinnen Americas, Laterigradæ, 1880.

PLATE XXIX, FIGURES 7–7*a*.

The female is 5 or 6$^{mm}$ long with the cephalothorax 2·5$^{mm}$ wide. The colors are black and gray on a white or yellowish ground, in irregular spots that vary in size in different individuals. There is usually a dark spot in the middle of the cephalothorax in front of the dorsal groove, and behind the eyes are four spots more or less run together and connected with smaller spots behind them. Along the sides of the thorax are four pairs of spots, the hinder pair largest. In the male these spots all connect together and the cephalothorax is often nearly black. The legs are covered with small dark spots and have larger spots near the ends of the joints and along the middle of the femur. In the male the femur, patella and tibia of the first and second legs are much darker than the other joints. Pl. XXIX, figs. 7, 7*a*.

The epigynum has the opening a long distance from the transverse fold and divided into two by a ridge that widens backward, Pl. XXIX, fig. 7*c*, much like the epigynum of *C. depressa*, of Europe.

The tibia of the male palpus has a large inferior process slightly bent inward at the end. The outer process is longer and the slender tip is on the inner side and s-shaped. Fig. 7*d*. The tube of the palpal organ is stout and has in the middle a thick portion, roughened like a file. The end of the tube is straight, not spiral as in *C. depressa*.

All over New England, on fences and under stones.

**Misumena vatia** Thorell = *Thomisus fartus* Hentz.

Adult females of this and of *aleatorius* are both named *vatia* by Keyserling.

PLATE XXX, FIGURES 1–1*g*.

The female of this species is the largest and best known of the genus. It is 8 or 10$^{mm}$ long and milk white, with sometimes a light crimson marking on each side of the abdomen. Pl. xxx, fig. 1. The cephalothorax is wide and high in front, the distance between the upper lateral eyes being twice their height. Fig. 1*b*. The sides of the cephalothorax are very light yellow or brownish, the dark color being most distinct in young spiders. The dark markings also extend between the eyes and around the sides of the head, but the front of the head has a white mark that widens below over the mandibles and above under the eyes and around the eyes of the upper row. This marking of the front of the head, Pl. xxx, fig. 1*b*, distinguishes this species from *aleatoria*.

The legs are white with light brown on the upper side of the first and second; or even this is absent in some individuals.

The epigynum has a deep rounded notch in the middle, the corners of which are prolonged backward over the spermathecæ. Each side of the large notch are two small ones. Pl. xxx, fig. 1*g*.

The male is only 3 or 4$^{mm}$ long, with the front legs about twice the length of the body. The males are strongly marked with dark reddish brown on a light ground. The cephalothorax is dark at the sides, while the front of the head around the eyes is white, as in the female. The abdomen has a dark stripe at the sides extending, sometimes, its whole length, or in other individuals not more than half as far. In the middle of the abdomen are two parallel dark marks or lines of spots, figs. 1*d*, 1*e*. The first and second legs have the femur dark brown, the patella dark on the outer half. The tibia dark at both ends, and the tarsus and metatarsus on the outer half. The palpi are light with the palpal organ brown. The third and fourth legs are light.

The male palpi are small. The tarsi are short and nearly as wide as long. The tibial process is smaller than in the other species and

has the littte hook on the end. Pl. xxx, fig. 1*f*. The bulb is round at the base and has a shallow notch at the distal end, over which is the short and small tube, twisted once at the end.

The young male has the form of the female with part of the markings of the male. The femora and palpi are light colored. Pl. xxx, fig. 1*c*.

The female and male from Menge's collection of Prussian spiders in the Museum of Zoology at Cambridge, are exactly like American specimens, except that the female has the hairs on the legs a little more distinct and the epigynum has the lateral openings larger and the middle one proportionally smaller.

Common all over New England, as far north as the White Mountains.

**Misumena aleatoria** = *Thomisus aleatorius* Hentz = *Runcinia Brendellii* Keys

Adult females are named by Keyserling *M. vatia*, and young females *Runcinia brendellii* in the Museum of Comp. Zoology at Cambridge.

PLATE XXX, FIGURES 2–2*d*.

The female of this species, Pl. xxx, fig. 2, is easily mistaken for *vatia*. It is smaller, the color is more yellow, from light straw color to orange, and it does not have the crimson stripes on the abdomen, though it occasionally has dark reddish brown marks in the same places and a double row of dark spots in the middle of the back. Pl. xxx, fig. 2*e*. The cephalothorax is light yellow or greenish, with the sides a little darkened with brown. The legs are usually yellow without markings, but sometimes, especially in individuals with spots on the abdomen, there are dark marks on the coxa and trochanter, patella, tibia, and metatarsus of the first and second legs. The front of the head is lower than in the other species and the distance between the upper lateral eyes is nearly three times their height. Below the eyes is a white stripe the lower corners of which are extended into lines over the mandibles and the upper corners into a very distinct white line that extends under the eyes and around the sides of the head. Pl. xxx, figs. 2*a*, 2*b*.

The epigynum (fig. 2*b*) has the notch less deep than in *vatia* and more open behind.

The male is only $3^{mm}$ long, but with the first and second legs $8^{mm}$ in length. The cephalothorax is much like that of the female, green with the sides dark brown. The abdomen is bright yellow. The first and second legs are dark brown without any markings, and the

palpi are brown but not quite as dark. The third and fourth legs are light yellow. The immature males have the front legs no longer than those of the female and only partly colored brown. Pl. xxx, fig. 2*d*.

The young of both sexes have the abdomen darker in the middle, with light stripes in the hinder half.

The male palpi, Pl. xxx, figs. 2*f*, 2*g*, are very small. The tibial process extends half the length of the tarsus and has a sharp point turned a little outward and just below the point a small hook turned upward. The palpal organ is much smaller than the tarsus, flat and circular with a short tube and without any other processes.

The abdomen of the female is a little flatter than that of *vatia*, straighter at the sides and more truncated behind.

Massachusetts and Connecticut.

**Misumena asperata** = *Thomisus asperatus* Hentz.

*Misumena georgiana* Keyserling, specimen in Mus. Comp. Zool., Cambridge; and *M. foliata* Banks.

PLATE XXX, FIGURES 3–3*e*.

This species does not grow as large as *vatia* and the color of the adult female is more generally yellow, sometimes deep yellow, but oftener pale and greenish. The legs are a little spotted with pale brown and more hairy than in *vatia*. The males and young are brightly colored with dull yellow and reddish brown markings, some of which are retained by the female until the last moult. A female half grown (Pl. xxx, fig. 3*a*) has the cephalothorax light in the middle with a brownish stripe each side covering half way to the edge. The abdomen has a dark red band on each side of the front half and in the middle a pattern in light brick red. The first and second legs are marked with dark red-brown spots on the end of the patella, both ends of tibia, and the end half of the metatarsus. The hairs are much longer than in the adult.

A female just before the last moult had lost entirely the markings of the legs (Pl. xxx, fig. 3*a*), and had the pale markings of the adult behind the eyes, while the abdomen showed the two side stripes broken into several spots and three pairs of spots on the hinder half darkest on the outer side, all brick red. This spider was perched on a plant of sorrel (*Rumex acetocella*) and its colors were exactly those of the flowers. The male has the colors of the young, but all deeper, the ground yellow or greenish and the mark-

ings dark reddish brown. The femora of legs one and two are spotted with dark red, thickest toward the front (fig. 3*b*).

The epigynum has a wide oval opening divided into two round smaller ones by a wide ridge on which is a thin raised line in the middle (fig. 3*c*).

The male palpus has a large complicated process on the outer side at the end of which is a small black point, hooked at the end. The tube of the palpal organ is wide and curved once around the end of the bulb (Pl. xxx, figs. 3*d*, 3*e*), ending on the outer side. The male palpus is much longer than that of *vatia* and more than twice as large as that of *aleatoria.*

The abdomen of the adult female has often gray or reddish markings along the sides or in the middle.

**Misumena oblonga** Keys., Spinnen Americas, 1880.

PLATE XXX, FIGURES 4–4*c*.

Male $2{\cdot}5^{mm}$ long. Front legs $8^{mm}$ long and very slender. Colors, in alcohol, greenish yellow on the cephalothorax and legs and white on the abdomen and around the eyes, with dark red markings. The front of the head is high, the distance between the upper lateral eyes being twice their height, as in *M. vatia*, but it does not have the white mark in front like that species. The eyes are all slightly raised on whitish tubercles. Pl. xxx, fig. 4.

The sides of the cephalothorax are slightly darkened and around its edge is a fine red line, short in some and in others extending its whole length, and there are also, in one specimen, red lines across the ends of the mandibles. The first and second legs have a short red ring at the end of the femur and the patella. The tibia has the distal half red and also a short ring at the base. The metatarsus is red for three-fourths its length, and the tarsus half its length. There are also a few red spots on the front of the first femur and at the ends of patella and tibia of fourth leg, but these spots are absent in one specimen. The cephalothorax, abdomen, and femora have stiff black hairs, standing wide apart.

The male palpi are as small as those of *aleatoria*, but shorter and more pointed, like those of *vatia.* The outer process of the tibia is much like that of *vatia*, but shorter and thicker, and the hook is placed more on the outer side. The tube is longer and straighter and more slender than in *vatia.* Pl. xxx, fig. 4.

The only female that seems to belong to this species is an immature specimen $4{\cdot}5^{mm}$ long. The color is pale yellow, darkened a

little at the sides of the cephalothorax and the ends of the legs. On the first and second legs there is a small dull red spot near the end of the tibia and a longer one on the metatarsus. The whole body is covered with long hairs arranged in rows. On the back of the abdomen there are about twenty rows of them nearly their length apart. On the legs they are in longitudinal rows a little nearer together. On the cephalothorax there is a middle row directed forward and another each side of it passing between the eyes. The other hairs of the cephalothorax point toward the middle. The body is smooth underneath, or with only very short hairs.

Males from Blue Hills, Milton, Mass.; New Haven and Simsbury, Conn.; young female from Brookline, Mass.; Washington, D. C., Dr. Fox.

## Philodromus.

Cephalothorax as wide as long, rounded at the sides and much narrowed in front as far back as the base of the palpi. The front row of eyes is much shorter than the upper row. The legs are long and slender, the second pair longest. The third and fourth legs are not much shorter than the first and second. The hinder legs are wide apart and the sternum extends backward between them. The feet have the claws turned backward and under them a thick bunch of long hairs, wide and flattened at the end. The abdomen is longer than wide and a little pointed behind. The males are but little smaller than the females and much longer legged.

The male palpi have two processes on the tibia, the usual hard hooked process on the outer side, and a flat, thinner one underneath.

*Philodromus vulgaris* is a common and distinct species. The others are all small and much alike, especially the males, and the difference between the sexes is so great that it is difficult to tell the male and female of the same species, and the males which are referred to *ornatus* and *lineatus* may prove to belong to other species.

**Philodromus vulgaris** Keys. = *Thomisus vulgaris* Hentz.

*P. vulgaris* Banks. *P. praelustris* and *P. signifer* Banks are probably of this species.

PLATE XXXI, FIGURES 1–1*g*.

Female, $8^{mm}$ long. Cephalothorax, $3^{mm}$ long and the same wide. Head less than half as wide as the middle of the cephalothorax. The abdomen is half longer than the cephalothorax and nearly as wide at the widest part. The colors are various shades of gray,

resembling closely those of old unpainted wood. The ground color is dirty white and the under side is almost entirely of this color. The dark portion of the back of the abdomen is darkest at the edges and does not entirely cover the abdomen, showing a lighter band around it, plainest around the hinder half. The principal middle markings are a long oval spot in front, pointed before and behind, and a herring-bone pattern behind. The legs are spotted with fine spots closest along the front of the femora and largest at the ends of each joint. Pl. XXXI, fig. 1. The males are darker and longer legged, as in most species, fig. 1*a*. The epigynum has two openings, between which is a ridge that widens behind and extends to the transverse fold. Usually this ridge has waved or irregular sides, as in fig. 1*f*, but in some the sides are rounded. Fig. 1*g*.

The male palpus is long with the tibia nearly twice as long as wide. The lateral process is as wide as long, square at the end, with the upper corner slightly hooked. The under process is smaller, rounded on the inner corner, and sharp pointed on the outer. Pl. XXXI, fig. 1*e*. The tarsus is twice as long as wide and pointed at the end. The tube is short and very thick at the base. It starts on the middle of the inner side of the bulb and extends around the end. Under the tip of the tube is a short fine process. Fig. 1*d*.

All over New England on houses and fences, but seldom on plants.

**Philodromus pictus**, new sp.=*P. rufus* (Walck.) Banks.

PLATE XXXI, FIGURES 2–2*e*.

Female 5 or 6$^{mm}$ long. Abdomen usually about twice as long as the cephalothorax. The widest part farther forward than in most species. Legs and palpi pale yellow with fine brown spots. Cephalothorax light yellow in the middle and reddish brown at the sides, covered with fine spots. Abdomen dull red at the sides and bright yellow in the middle with a dark greenish marking in the middle of the front half and two dark marks behind it on the hinder half. Pl. XXXI, fig. 2. The eyes are surrounded by distinct light rings. In some specimens, usually immature, the abdomen has a more distinct yellow and red pattern. Fig. 2*a*.

The male has the cephalothorax and legs darker and the abdomen less bright red and yellow than the female, and sometimes gray and iridescent. In alcohol it shows a more distinct herring-bone pattern on the hinder half. Fig. 2*b*.

The male palpus has the tibia nearly as long as the tarsus. The lateral process is unusually long and the upper corner has a sharp

hook. Fig. 2*g*. The under process is very thin and about as long as the lateral. The tarsus is widened on the inner side and the tube is long and slender, starting near the base of the bulb. The eyes are close in both sexes. Figs. 2*c*, 2*d*, 2*e*.

A very common species all over New England.

**Philodromus ornatus** Banks.

=*P. minusculus* Banks and *P. placidus* Banks.

PLATE XXXI, FIGURES 3–3*b*.

The female of this species is small and very distinctly marked with dark brown on a white ground. The middle of the cephalothorax is white and the sides brown nearly to the edge. The abdomen is white with a distinct brown band each side from the front more than half its length. Pl. XXXI, fig. 3. Sometimes there is also an indistinct brownish pattern in the middle, but this is usually absent in adults and the middle is entirely white. The coxæ are brown and the rest of the legs white, except a little brown at the ends of the joints. Under the abdomen the lateral brown bands extend backward and meet across the spinnerets. The abdomen is nearly as wide as long and widest across the hinder half.

The male which I suppose belongs to this species has the legs and cephalothorax orange brown, darker at the sides of the thorax and toward the ends of the legs. The abdomen is dark reddish-brown, strongly iridescent with red and green in a bright light. In alcohol it shows brown markings at the sides similar to those of the female, and also indistinct angular marks in the middle of the hinder half. The palpi are long, but the tibia is little longer than wide and narrower than the patella. The outer process is small and the under process wide and long, extending over the bulb a third its length. Pl. XXXI, figs. 3*a*, 3*b*. The tarsus is widest across the middle and straight on the outer side. The tube is very long and slender beginning at the base of the bulb near the under tibial process. Fig. 3*a*.

All parts of New England; Ithaca, N. Y., Banks.

**Philodromus lineatus**, new sp.

PLATE XXXI, FIGURES 4–4*c*.

The female of this species is a little larger than *ornatus* and the brown markings are lighter, and in life, or when freshly killed, purplish in the lighter parts. The markings are less distinct than in *ornatus*, the brown and white running into each other. The abdo-

men has a brown band each side, often broken into several spots, and a brown band in the middle extending back half its length, behind which are several lighter marks. Between these are many oblique lighter markings and rows of spots. The legs are very light grayish brown, darker toward the ends of the joints. Pl. XXXI, fig. 4. The upper middle eyes are farther apart than in *ornatus*.

The supposed male of this species resembles closely the last, but is a little larger and has much larger palpal organs. The tibia is longer than wide. The outer process is very short and pointed obliquely outward. The under process is long and wide. Fig. 4*b*. The tarsus is nearly as wide as long, and the palpal organ nearly round. The tube begins on the outer side beyond the end of the under process and extends around the inner side and outer end. Fig. 4*c*.

All over New England. Not found by Banks at Ithaca, N. Y.

## Philodromus bidentatus, new sp.

PLATE XXXI, FIGURES 5-5*b*.

Of this species I have only males, one from New Haven, Conn., and two from Mt. Tom, in the central part of Massachusetts. They are $3^{mm}$ long and in their general appearance agree with the other species. The New Haven specimen is pale and has the markings very distinct, while the others are dark orange brown, like most male *Philodromus*, after a long time in alcohol. The markings of the legs are more in irregular patches of gray and less in fine spots than in most species, and the dark middle line of the cephalothorax is unusually distinct. The abdomen has the usual markings. Fig. 5. The male palpi have the tibia shorter than the patella and widened at the distal end. The lateral process is stout and has two teeth at the end. Pl. XXXI, fig. 5*b*. The under process is longer, but small compared with other species. Fig. 5*a*. The tube is shorter than in *pictus*, beginning near the middle of the side of the bulb.

## Philodromus brevis, new sp.

PLATE XXXII, FIGURES 2-2*d*.

Males, $2{\cdot}5^{mm}$ long. The color of both specimens is dark. The cephalothorax does not have the usual light area in the middle, but only a lighter spot just in front of the dorsal groove. The rest of the cephalothorax is nearly uniform in color. Fig. 2.

The male palpi have the tibia short and straight, not widened at the ends. The lateral hook is long and stout and slightly curved

down at the end. Fig. 2*d*. The inferior process is short and wide. The tarsus is short and blunt. The tube is very short, not half the length of the tarsus, and nearly straight. Pl. XXXII, fig. 2*c*.

Two males only, from Readville, Mass.

**Philodromus robustus**, new sp.

PLATE XXXII, FIGURES 1–1*a*.

Male, $4^{mm}$ long. Legs and palpi stouter and more hairy than in the other species, and the mandibles longer. The specimen is much faded and there are no traces of markings on the legs. The abdomen shows the usual markings and the cephalothorax had the middle light colored with a dark line, widening toward the head.

The palpi have the tibia longer than wide and about as long as the patella. The outer process is slender and short, turned obliquely outward and blunt at the tip. Fig. 1*a*. The under process is thin but hard and as wide as long. The tarsus is as wide as long and the palpal organ nearly round. The tube is slender and extends half way around the bulb.

Beverly, Mass.

## Tmarus E. Simon, 1864.

Cephalothorax widest across the middle, farther forward than in most of the family. Front of the head and mandibles inclined far enough forward to be seen from above. Lateral eyes of both rows on large round tubercles. Middle eyes forming a quadrangle longer than wide and widest behind. Abdomen longer than wide, widest across the hinder half and high, and pointed behind with the point in some species prolonged into a tubercule of various shapes.

**Tmarus caudatus** Keys. = *Thomisus caudatus* Hentz.

PLATE XXXII, FIGURES 3–3*d*.

Female, $6^{mm}$ long. Cephalothorax, $2^{mm}$ long and as wide at the widest part. The abdomen is as narrow as the cephalothorax in front and widens backward to nearly twice that width. Fig. 3. The abdomen rises from the front to a point over the spinnerets, where it forms a blunt conical point. Fig. 3*a*. The first and second legs are nearly equal and much longer than the third and fourth. The mandibles are inclined forward, their basal half is nearly straight and the ends narrowed. The colors are gray and white, resembling light individuals of *Philodromus vulgaris*. The legs are

spotted with a few black dots, largest and closest on the front pair. The markings of the cephalothorax radiate indistinctly from the dorsal groove. The abdomen is covered with fine gray spots and has three or four pairs of darker lines across the hinder half. Pl. XXXII, fig. 3.

The males differ little from the females except in the smaller abdomen.

The male palpus has a short and blunt lateral process widened in the middle and thin and sharp at the end. Figs. 3*b*, 3*c*. The tarsus is wide and the palpal organ round and without any processes in the middle. The tube is slender, passing almost entirely around the bulb and supported at the end by a soft appendage with a hard sharp point. Figs. 3*b*, 3*c*. The epigynum has a small semicircular opening. Fig. 3*d*.

A common species in Massachusetts and Connecticut.

### Ebo Keyserling, 1883.

These spiders have a general resemblance to *Philodromus*. The whole body is flattened and wider than in that genus, the head is more rounded and the two rows of eyes more nearly of the same length. The legs of the second pair are much longer than the others.

### Ebo latithorax Keys., Zool. bot. ges., Wien, 1883.

PLATE XXXII, FIGURES 4–4*d*.

In this species the second pair of legs is more than twice as long as either of the others and has the claws of these legs much smaller than the others. The cephalothorax is wider than long and much flattened. The head is rounded in front. The mandibles are small and turned backward under the head. The eyes are arranged as in *Philodromus*, but there is less difference between the length of the two rows and the front middle eyes are larger than the others. Pl. XXXII, figs. 4*b*, 4*c*, 4*d*. The abdomen is as wide as long and a little pointed behind. Figs. 4, 4*a*.

The length is 2 to 2·5$^{mm}$. The color is light yellow with brownish markings on the cephalothorax and abdomen. On the cephalothorax are several radiating lines of spots running from the dorsal groove to the legs, and three shorter lines extending forward half way to the eyes. The abdomen has two dark spots at the sides in front and a line in the middle and an indistinct herring-bone pattern on the hinder half.

There is little difference between the male and female. The male palpus is slender and the palpal organ small. The tibia is twice as long as wide and has a short process on the outer side. The tarsus is slightly widened across the middle and the palpal organ is oval, about twice as long as wide. The tube is short and curved in a half circle across the end of the bulb. Figs. 4*e*, 4*f*.

Medford, Mass.; Providence, R. I.; Long Island, N. Y., in N. Pike's collection. The only males I have seen are in the latter collection.

### Tibellus Simon, 1875.

Cephalothorax longer than wide. Abdomen very long and slender, narrower than the cephalothorax, and straight at the sides. Legs slender with long spines, second pair longest, and the fourth nearly as long as the first. Eyes on the top of the head. Both rows curved and the front row about half as long as the upper. The femur and patella of the female palpi a little thickened.

**Tibellus duttonii** Keys. = *Thomisus duttonii* Hentz.

PLATE XXXII, FIGURES 5–5*c*.

Female, 8 or 10$^{mm}$ long, the abdomen variable in length and thickness. The color is light yellow with light brown markings. The cephalothorax has a middle brown stripe and one on each side, all indistinct. The abdomen has two black spots on the hinder half and a light brown middle spot at the front end extending back half the length of the abdomen and ending in a point, or sometimes continuing the whole length. The abdomen is a little pointed behind and extends back far enough to cover the spinnerets. Pl. XXXII, fig. 5.

The male is a little more slender than the female. The male palpus is bent downward. The patella and tibia are both short and the tibia shorter on the under side than above, so that the tarsus joins it obliquely. Fig. 5*a*. The tibial process is short and blunt. The tube is stout and black and twisted at the tip and has beside it a flat process of about its own length. Fig. 5*b*. I have not been able to find the spider whose palpus was figured by me on plate 20 of the reprint of Hentz's spiders of the United States in 1875. All that I have seen since have the slender portion of the tube longer, as here figured. The epigynum is very far forward and has its opening behind over the transverse fold. Fig. 5*c*.

Massachusetts; common in the White Mountains and Northern New York.

### Thanatus C. Koch.

Cephalothorax as wide as long in males and almost as wide in females, resembling that of *Micrommata* and *Dolomedes*. The front row of eyes is much shorter than the upper row and both rows are curved. Pl. XXXII, fig. 6. The abdomen is oval and little flattened, but not as long and slender as in *Tibellus*. The fourth legs are as long as the second or longer, which is unusual in this family.

### Thanatus lycosoides, new sp.

PLATE XXXII, FIGURES 6–6c.

This species is very near *T. coloradensis* Keys., and may be the same. It is smaller than the spiders described by Keyserling in both sexes, but the proportions are the same, and the male palpi almost like those of *coloradensis*, being a little more slender and having a sharper tibial process. Keyserling's specimens were all from Colorado and I have not seen any of them.

The female is 6 to $8^{mm}$ long. Cephalothorax of largest specimen, $4^{mm}$ long and nearly as wide. Length of legs, 4, 2, 1, 3. The legs are slender and tapering, the fourth pair as long as the second or longer. The colors are white and brown covered with brown and gray scales and scattered brown hairs, and the whole appearance is much like *Lycosa* or *Dolomedes*. The middle of the cephalothorax is light from the eyes backward, and on each side is a dark brown band. The abdomen has a distinct brown spot extending from the front end beyond the middle, and pointed at both ends. This spot is found in several species of the genus. The legs are indistinctly marked on the femur and tibia with longitudinal brown lines. Pl. XXXII, fig. 6. The epigynum is close to the transverse fold and is divided by a flat ridge, widest in front. The openings are long and narrow and covered by a convex brown shell, each side opening widest toward the transverse fold. Fig. 6*c*.

The male is, as usual, smaller and longer legged and resembles more *Ocyale* and *Philodromus*. The male palpus is somewhat like that of *duttonii*, but shorter and stouter, and it has a longer and larger tibial hook. The palpal organ is large and extends beyond the tarsus on the outer side. The tube is short and slender and a little curved. Over the tube is a small, flat, soft appendage. Figs. 6*a*, 6*b*.

Wenham, Annisquam, Dedham, Mass.; and Mt. Carmel, Hamden, Conn.

## EXPLANATION OF PLATES.

### Plate XXVIII.

Figure 1.—*Xysticus limbatus* Keys., female, × 4; 1*a*, male, × 4; 1*b*, young female with more distinct markings; 1*c*, maxillæ and labium; 1*d*, 1*e*, 1*f*, opening of epigynum of three different spiders; 1*g*, male palpus; 1*h*, tibia of male palpus from above.

Figure 2.—*Xysticus gulosus* Keys., female, × 4; 2*a*, male, × 4; 2*b*, male palpus; 2*c*, epigynum.

Figure 3.—*Xysticus stomachosus* Keys., female, × 4; 3*a*, male, × 4; 3*b*, 3*c*, male palpus; 3*d*, epigynum.

Figure 4.—*Xysticus nervosus*, female, × 4; 4*a*, male, × 4; 4*b*, epigynum; 4*c*, tarsus and tibia of male palpus; 4*d*, under side of male palpus.

### Plate XXIX.

Figure 1.—*Xysticus triguttatus* Keys., female, × 4; 1*a*, male, × 4; 1*b*, front of head of female; 1*c*, epigynum; 1*d*, male palpus.

Figure 2.—*Xysticus graminis*, male, × 4; 2*a*, male palpus; 2*b*, dorsal markings of female, × 4.

Figure 3.—*Xysticus formosus*, male, × 4: 3*a*, male palpus.

Figure 4.—*Xysticus quadrilineatus* Keys., female, × 4; 4*a*, epigynum.

Figure 5.—*Xysticus inornatus*, female, × 4; 5*a*, front of head of female; 5*b*, epigynum.

Figure 6.—*Oxyptila cinerea*, male, × 4; 6*a*, male palpus.

Figure 7.—*Coriarachne versicolor*, female, × 4; 7*a*, male, × 4; 7*b*, front of head of female; 7*c*, epigynum; 7*d*, male palpus.

### Plate XXX.

Figure 1.—*Misumena vatia* Thor., female, × 4; 1*a*, cephalothorax, enlarged; 1*b*, front of head; 1*c*, young male, × 4; 1*d*, 1*e*, adult males of different sizes, × 4; 1*f*, male palpus; 1*g*, epigynum.

Figure 2.—*Misumena aleatoria*, female, × 4; 2*a*, front of head; 2*b*, top of head; 2*c*, male, × 4; 2*d*, young male, × 4; 2*e*, young female with dark markings, × 4, from one in the collection of the Boston Soc. Nat. Hist.; 2*f*, 2*g*, male palpus; 2*h*, epigynum.

Figure 3.—*Misumena asperata*, female, × 4; 3*a*, young female, × 4; 3*b*, male, × 4; 3*c*, epigynum; 3*d*, male palpus; 3*e*, side of male palpus, less enlarged.

Figure 4.—*Misumena oblonga* Keys., male, × 4; 4*a*, 4*b*, male palpus; 4*c*, young female, × 4.

### Plate XXXI.

Figure 1.—*Philodromus vulgaris* Keys., female, × 4; 1*a*, male, × 4; 1*b*, first foot, outer side; 1*c*, first foot, inner side; 1*d*, male palpus; 1*e*, tibia of male palpus, outer side; 1*f*, usual form of epigynum; 1*g*, epigynum of a female from Providence, R. I.

Figure 2.—*Philodromus pictus*, female, × 4; 2*a*, dorsal markings of a younger female; 2*b*, male, × 4; 2*c*, front of head of female; 2*d*, top of head of male; 2*e*, front of head of male; 2*f*, 2*g*, male palpus.

Figure 3.—*Philodromus ornatus*, female, × 4; 3*a*, 3*b*, male palpus; 3*c*, tibia of male palpus from above; 3*d*, epigynum.

Figure 4.—*Philodromus lineatus*, female, × 4; 4*a*, sternum; 4*b*, side of male palpus; 4*c*, male palpus, under side.

Figure 5.—*Philodromus bidentatus*, dorsal markings of light colored male, enlarged eight times; 5*a*, 5*b*, male palpus.

PLATE XXXII.

Figure 1.—*Philodromus robustus*, palpus of male; 1*a*, outer side of tibia of male palpus.

Figure 2.—*Philodromus brevis*, markings of cephalothorax of male, × 8; 2*a*, top of head; 2*b*, front of head; 2*c*, 2*d*, male palpus.

Figure 3.—*Tmarus caudatus* Keys., female, × 4; 3*a*, side of abdomen of female; 3*b*, 3*c*, male palpus; 3*d*, epigynum.

Figure 4.—*Ebo latithorax* Keys., female, × 4; 4*a*, male, × 4, from a specimen in N. Pike's collection of Long Island spiders; 4*b*, 4*c*, 4*d*, head of female; 4*e*, 4*f*, male palpus.

Figure 5.—*Tibellus duttonii* Keys., female, × 4; 5*a*, 5*b*, male palpus; 5*c*, epigynum.

Figure 6.—*Thanatus lycosoides*, female, × 4; 6*a*, 6*b*, male palpus; 6*c*, epigynum.

## XXII.—The Marine Nemerteans of New England and Adjacent Waters. By A. E. Verrill.

The following article is intended as a descriptive catalogue of all the Nemerteans of the North-eastern Coast of North America that have been observed with enough care to permit me to give a description presumably sufficient to enable ordinary observers to identify the species when seen living. Therefore all my own descriptions, herein given, have been made from living specimens, except in a few special instances, which are, in each case, particularly stated.

As a rule, undetermined alcoholic specimens of Nemerteans, unaccompanied by notes on their forms and colors while living, cannot be identified with certainty unless they belong to genera containing very few and widely differing species. To distinguish the numerous species of *Amphiporus*, *Tetrastemma*, *Lineus*, etc., with alcoholic specimens alone, would be a hopeless task, at least in the present state of our knowledge of these groups. Possibly, when all the known species shall have been studied thoroughly by means of microscopic sections, it may be possible to distinguish many of the species by means of such sections of preserved specimens, but that will be a condition possible only in the distant future, and in any case would require much time and labor.

Exceptional cases are, however, not uncommon in which some prominent feature may be preserved in the alcoholic specimens sufficiently well to enable the species to be recognized with certainty. Thus, among the *Enopla*, the stylets of the proboscis are frequently characteristic in form or number. The ocelli, often visible in alcoholic specimens, may also be characteristic. In a few cases, even the characteristic colors may be preserved many years in alcohol, and still better in glycerine. I have specimens of *Amphiporus angulatus* (*Stimpsoni*), preserved in alcohol twenty years ago, in which the dark purple color of the body and the characteristic white patches on the sides of the head are still very distinct. These specimens have, however, been kept in dark drawers; those that were exposed to light faded many years ago.

In consequence of the difficulty or impossibility of identifying alcoholic specimens, I have, in this article, made very little use of a

large part of the vast collection of American Nemerteans preserved in the Museum of Yale University, and including those collected by myself and others during the explorations carried on from 1871 to 1887 by the United States Fish Commission, under the direction of the late Commissioner, Professor S. F. Baird.

These collections include several thousands of specimens, filling more than a thousand bottles and jars. They represent very fully the Nemertean and Planarian fauna of the coast, from Cape Hatteras to Labrador, and from high-water mark to 2000 fathoms. Fortunately I personally identified and labelled when captured a large number of those specimens that belonged to described species, and made copious descriptions and sketches of most of the unfamiliar forms that came, while still living, under my observation during all the sixteen seasons spent on the work of the U. S. Fish Commission, as well as during several summers (1864 to 1870) spent in independent researches in the waters of the Bay of Fundy and elsewhere. But there were many specimens, especially deep-water forms, that I did not see until they had been placed in alcohol. Most of those are entirely omitted from this paper. Probably they include a number of additional species.

Many of the general figures accompanying this article were made from life by Mr. J. H. Emerton and Mr. J. H. Blake, under my direction, for the U. S. Fish Commission. For the privilege of using these drawings for the present purpose, I am indebted to the late commissioner, Professor Baird, this article having been in preparation before his death. Other figures have been drawn by myself for this paper. Numerous figures, taken from my own field-notes and rough sketches, have been copied and put into shape by my son, A. H. Verrill, under my personal supervision. The latter are, therefore, quite as reliable as the former ones. A few anatomical figures (on Pl. XXXIX) have been copied from the works of McIntosh* and Hubrecht,† in order to illustrate more fully some of the differences between the orders and sub-orders of Nemerteans.

It was originally a part of my intention to have included numerous anatomical details of our native species, based on new preparations and studies, but various circumstances have compelled me to defer that portion of the subject to a future time. Such details are, however, less essential in the case of our Nemerteans than they

* A Monograph of the British Annelids. Part I. The Nemerteans. Ray Society, 1873.

† Voyage of the Challenger, vol. xix.

otherwise would have been, because many of our native species are closely allied to, and several others are identical with, some of those that have been well studied anatomically by McIntosh and other European writers.

Nemerteans are almost universally present on our shores, between tides at all levels, from near high-water (*Lineus socialis*) downward. They are also to be found, by dredging, at all depths down to 1000 fathoms or more, but are much more abundant in shallow water (1 to 60 fathoms) than at greater depths. They occur on all kinds of bottoms, but are usually more abundant in soft and partially organic mud than elsewhere. But in rather shallow water, on some hard bottoms overgrown with ascidians, hydroids, and sponges they are often very abundant, especially in the Bay of Fundy. The littoral Nemerteans occur in greater numbers and of more numerous species on the rocky shores of the Bay of Fundy, and especially in Eastport harbor, than in any other localities where I have collected them.

On sandy shores, also, there are nearly always several species living buried in the sand, to be found easily by the use of a spade. These sand-dwelling forms include the largest species of *Cerebratulus*, which are, perhaps, the largest of all Nemerteans.

## NEMERTINA.

The Nemertinea may be characterized as follows:

Smooth, ciliated, often bright colored, and mostly marine worms, destitute of external paired appendages, usually with a long and somewhat flattened body, often almost linear; without definite body-cavity. Muscular walls of body thick and complex, not segmented.

Head not very distinct from the body; mouth ventral, beneath the head, or subterminal, without teeth or jaws.

Intestine large, usually straight and furnished with many short, lateral, saccular, often lobed appendages; anus posterior.

A long, tubular, dorsal proboscis is contained in a special muscular sheath, which is filled with a corpusculated fluid and situated above the intestine but entirely separated from it. The proboscis can be protruded by eversion from a special aperture at the front of the head.

Two pairs of cephalic ganglions are present; they are united transversely by an upper and a lower commissure, between which pass the proboscis and its sheath. Most species have ciliated pits or sacs connected with the posterior ganglions by ducts leading from

fossæ or grooves on the sides of the head. They are probably olfactory organs. Two large lateral nerves run back from the lower cephalic ganglions; often there is also a smaller median dorsal nerve trunk, and in many there is a continuous nervous plexus between the muscular layers of the body-wall.

Vascular system closed; a main longitudinal vessel runs along each side and usually a median dorsal one is situated above the intestine; the blood is usually colorless, rarely red.

A paired nephridial system, consisting of ducts and tubules variously arranged, is usually present in the œsophageal region.

The sexes are almost always separate and nearly all the species are oviparous. Reproductive organs are very simple and similar in both sexes, consisting of simple saccular ovaries or spermaries, situated along the sides of the body, usually between the lateral saccules of the intestine. External genital openings are mere pores in the body-wall.

Development is usually direct, but sometimes with a metamorphosis through a *Pilidium*, or free swimming larval form, very peculiar in structure. (Plate XXXIX, figures 1 to 6).

## Order I, ENOPLA.

*Enopla* M. Schultz; McIntosh, Brit. Annelids, Part I, Nemerteans, pp. 36, 43, 134.
*Hoplonemertini* Hubrecht; Carus, Fauna Mediterraneæ, p. 163, 1884.
*Hoplonemertea* Hubrecht; Voy. Challenger, vol. xix, p. 15.

Proboscis divided into three distinct regions (Plate XXXIX, figures 7, 8, 9); the first is evertible and tubular; the middle region (woodcut 1), is furnished with a hollow muscular bulb and a complex armature, consisting of a central calcareous stylet (c), or a toothed plate, usually accompanied by two or more lateral chambers containing small, pin-like, free spines or stylets (figures 7, 7*a*, and woodcut 1, D, D′). The central cavity of the bulb and the lateral styliferous chambers communicate with the anterior evertible chamber of the proboscis by means of ducts. Proboscis-pore is either terminal, at the end of the snout, or subventral.

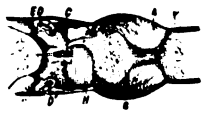

Fig. 1. Armature of proboscis of *Amphiporus lactifloreus;* A, muscular bulb; B, its cavity; C, central stylet; D, D′, lateral stylet-sacs: E, duct of D; H, muscular band, (after McIntosh).

The mouth is small and inconspicuous when contracted, situated beneath the end of the snout and in front of the cephalic ganglions, often in close connection with the proboscis-pore.

Lateral longitudinal cephalic slits are wanting and are generally replaced by shallow, transverse or oblique, ciliated grooves or fossæ, connected by narrow ducts with small sacs (probably olfactory in function) that connect them with the posterior ganglions.

Cephalic ganglions rounded, the upper or anterior ones closely united to and largely covering the lower ones. Lateral nerves arise from the posterior ends of the inferior ganglions and run back within the inner muscular layer of the body-wall.

Ocelli various, often numerous; sometimes wanting.

Three large, longitudinal vascular trunks are well developed; a vascular loop in the head.

Intestine large and straight, sacculated. Muscular walls of the body consist mainly of two layers, an outer circular, and an inner longitudinal one.

The young, so far as known, undergo no marked metamorphosis.

The species are chiefly marine; a few fresh-water and terrestrial species are known.

## Family, Amphiporidæ McIntosh (restr.)

Body moderately elongated. Proboscis with a thick, tubular, evertible anterior portion, its walls consisting of about seven layers, the inner surface (or outer when protruded) thickly covered with papillæ; middle region furnished with a simple central stylet and generally two or four lateral chambers containing pin-shaped stylets, but the lateral sacs are sometimes wanting; posterior region tubular, with two muscular layers, an outer circular and an inner longitudinal layer. Œsophagus with a dilated and plicated anterior portion in the head.

The family *Tetrastemmidæ* Hubr. is here included. I can find no characters that seem to me sufficient to warrant even a sub-family distinction between *Tetrastemma* and *Amphiporus*.

On the other hand, I would separate *Drepanophorus* Hubr. as a separate family, Drepanophoridæ, characterized by having the central armature of the proboscis in the form of a lamina or plate, bearing several stylets or denticles; by the numerous styliferous sacs; and by the presence of lateral cæcal sacs connected with the sheath of the proboscis.

**Amphiporus** Ehrenberg, 1831, McIntosh, *non* Dies., *nec* Œrsted.

*Ommatoplea* Ehr., Symbolæ Physicæ, 1831.
*Omatoplea* Diesing, Syst. Helm., vol. i, p. 248, 1850.
*Polystemma* Ehr., 1831; Œrsted, Naturhist. Tidssk., iv, p. 579, 1844.
*Polia* Quatr. (*pars*), Ann. des Sci. Nat., vi, p. 201, 1846, *non* Delle Chiaje, 1841.
*Cosmocephala* Stimpson, Prodromus, in Proc. Acad. Nat. Sci., Philad., vol. ix, p. 165 [21. sep. copy]. 1857.
*Polina* Stimpson, op. cit., p. 165.
*Ophionemertes* Verrill, Amer. Journ. Sci, vol. vii, p. 45, 1873; Proc. Amer. Assoc., 1873, p. 389, 1873.

Body only moderately elongated, in some species slender, in others stout; usually strongly convex dorsally and with rounded sides.

Head often distinct from the narrowed neck, but in other cases of the same breadth as the body and without any definite limitation.

Transverse or oblique ciliated fossæ or shallow grooves, two of them connected with the ciliated sensory ducts, are, apparently, always present, though often very indistinct; usually there is a pair at the back of the head and nearly in line with, or just behind, the posterior ocelli and the ganglions; the other pair, situated in front of the ganglions, is usually less distinct and may be easily overlooked, and is perhaps absent in some species. One or both pairs of fossæ may meet on the dorsal line in certain species.

Ocelli usually numerous, variously arranged; perhaps the most common or typical arrangement is that of two anterior groups and two posterior or cerebral clusters, but either pair may be lacking, or the two groups may blend, and sometimes no ocelli are visible.

Proboscis-pore terminal, or sometimes sub-terminal, just under the tip of the snout. Proboscis large and long. Central armature a simple, sharp stylet with thick base; lateral stylet-sacs usually two, each with two to four, or more, pin-shaped stylets.

Mouth far forward, usually united with the proboscis-pore, and therefore not visible in contraction.

The numerous species belonging to this genus* were distributed

* Many authors, of whom a few are indicated in the synonymy, have used *Ommatoplea* Ehr. as the name of this genus, and on many accounts it seems to me that it would have been better to have continued that usage. McIntosh, in his monograph, has, however, seen fit to change the name to *Amphiporus* (of the same date) for reasons that are, to say the least, of questionable validity,—mainly because somebody *may* hereafter discover that the "type" of *Ommatoplea* is of a different genus, though he gives no reason for supposing that to be the case. In this instance long usage

among a large number of genera by the earlier writers, especially by those who did not observe the armature of the proboscis, or did not consider it of importance.

In general, it is impossible to distinguish the species of this genus from other genera without an examination of the proboscis and its armature. Hence, no doubt, there are many still unrecognized species of the genus that were formerly described under other genera, from various foreign countries. I believe, however, that all the species hitherto described or mentioned as found on our coast are included in the following list, together with several that appear to be undescribed.

Œrsted adopted *Polystemma* for this genus, and placed under it two typical species; *P. roseum* and *P. pulchrum*. At the same time he restricted *Amphiporus* to the genus named *Nemertes* (new sense) by McIntosh, giving its essential character (a small proboscis) and naming *A. Neësii* as the type.

### *Subgenera of Amphiporus.*

The genus may be conveniently divided into several groups or subgenera based primarily on the arrangement of the ocelli and nerves, as follows:

I. Ocelli form four or more distinct groups; the two cerebral groups are distinct from the anterior ones.—*Ommatoplea*, subgenus.

1*a*. Anterior ocelli do not form curved rows parallel with the lateral margins of the head.

*Amphiporus angulatus* (Fabr.).
*A. multisorus* V., sp. nov.
*A. heterosorus* V., sp. nov.
*A. tetrasorus* V., sp. nov.

1*b*. Anterior groups of ocelli form curved rows parallel, at least in part, with the sides of the head.—*Polystemma* Œrs.; *Polina* Stimp.

*A. roseus* (Müller).
*A. lactifloreus* (Johnst.).
*A. ochraceus* V.
*A. glutinosus* V.
*A. griseus* (Stimp.).

would have justified him in not making the change before there was any proof of the necessity for doing so.

The change having been made in so important a work, has been generally adopted by later European authors, and I have, therefore, followed their example in this article, for uniformity of nomenclature in this group is at present of paramount importance.

II. Only two distinct groups of ocelli, anterior or sub-lateral; cerebral groups obscure or wanting.—*Cosmocephala* Stimp.

2*a*. Only anterior groups of ocelli are evident.
*A. frontalis* V., sp. nov.

2*b*. Only median lateral groups of ocelli are present; or else the median and cerebral groups are blended.
*A. mesosorus* V., sp. nov.

III. Ocelli form only elongated lateral rows more or less parallel with the sides of the head; cerebral groups are not distinct from the others. Body slender.—*Ophionemertes* Verrill.
*A. cruentatus* V.
*A. virescens* V.
*A. agilis* V.

IV. Only a single pair of anterior ocelli are present.—*Dichilus* Stimp.
*A. bioculatus* McInt.

V. Ocelli indistinct or absent.—*Naredopsis* Verrill, sub-gen. nov.
*A. cœcus* V., sp. nov.

VI. Ocelli doubtful, forming at least a pair of antero-lateral groups (perhaps others that are not observed).
*A. thallius* V., sp. nov.

VII. Cerebral groups of ocelli(?) alone observed (perhaps anterior ocelli overlooked).—*Nareda* Girard.
*A. superbus* (Gir.)

## Amphiporus.

*Analytical Table of species based on the arrangement of the ocelli.*

A. Ocelli present.
B. Ocelli numerous, arranged in groups.
C. Ocelli arranged in four groups; two cerebral, near the ganglions, and two anterior or antero-lateral.
D. Anterior clusters of ocelli transverse, at the front margin of the head: posterior groups roundish.
*a*. Anterior groups transversely oblong or partly double. *A. angulatus.*
*aa*. Anterior groups each divided into three subordinate clusters. *A. multisorus.*
DD. Anterior clusters are not transverse at the front margin.
*b*. Anterior clusters subdorsal, not parallel with the margins of head.
*c*. Anterior clusters are triangular with the acute angle backward. *A. heterosorus.*
*cc*. Anterior clusters are oblique rows parallel with the posterior ones. *A. tetrasorus.*
*bb*. Anterior clusters lateral or sublateral, curved or crescent-shaped, anteriorly partly parallel with the margins of head.
*e*. Posterior groups form round or angular close clusters.
*f*. Anterior groups are large, composed of several rows posteriorly, and nearly blend with posterior groups. *A. roseus.*

*ff.* Anterior groups are more simple, distinctly separated from the posterior clusters, composed of one or two rows, and regularly curved. *A. lactifloreus.*

*ee.* Posterior groups of few ocelli, which do not form close clusters.

*g.* Posterior groups consist of one, two, or rarely three, oblique pairs of ocelli on each side.

*h.* Posterior pairs usually two on each side, convergent backward. *A. ochraceus.*

*hh.* Posterior pairs of ocelli divergent backward. *A. glutinosus.*

*gg.* Posterior groups linear, each of about four ocelli. *A. griseus.*

CC. Ocelli arranged in only two distinct clusters.

*a.* Clusters transverse, short, in front of ganglions.

*b.* Clusters transverse, near front margin of head. *A. frontalis.*

*bb.* A large angular cluster on each side of the middle of the head. *A. mesosorus.*

*aa.* Clusters of ocelli elongated, lateral, parallel with the margins of the head.

*c.* Ocelli forming a simple row on each side of head. *A. cruentatus.*

*cc.* Ocelli in double or triple rows.

*d.* Ocelli in two or three nearly parallel rows extending back of ganglions. *A. virescens.*

*dd.* Rows of ocelli broad, terminating at the ganglions. *A. agilis.*

BB. Ocelli two only, near the front of head. *A. bioculatus.*

AA. Ocelli wanting or indistinct. *A. cœcus.*

Species not included in the above table :

*Amphiporus thallius.* Ocelli doubtful ; only front groups observed.

*Amphiporus* (?) *superbus.* Ocelli doubtful ; apparently two cerebral groups only.

## Amphiporus angulatus (Fabr.) Verrill.

*Fasciola angulata* O. Fabr., in O. F. Müller, Verm. Terrest. et Fluv., i. pp. 58, 1774.

*Planaria angulata* O. Fabr., in Müller, Zool. Danic., Prod., p. 221, 1776. (Communicated by O. Fabricius, t. Müller).

*Planaria angulata* O. Fabr., Fauna Grœnlandica, p. 323, 1780.

*Omatoplea Stimpsonii* Girard, in Stimpson, Invert. of Grand Menan, p. 28, pl. 2, fig. 18, 1853.

*Amphiporus Stimpsoni* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., vol. ii, p. 184, 1879; Check List Marine Invert. Atlantic Coast, p. 12, 1879; Bulletin U. S. Nat. Museum, No. 15, p. 143, 1879 (from Cumberland Gulf).

*Amphiporus Fabricii* Levinsen, Bidrag til Kundskab om Grœnlands Turbellariefauna, p. 38, 1879, from Vidensk. Meddel. fra den naturh. Foren. i Kbhvn., 1879-80, p. 200.

PLATE XXXIII, FIGURES 1, 1*a*, 2.

Body large and stout, only moderately elongated in extension ; back convex, sides well rounded, lower surface flattened. The body is very changeable in form and can contract into a short, thick,

oblong mass; the posterior end is often the broadest part, but frequently in extension the breadth is nearly uniform throughout most of the length; posterior end obtuse. Head usually more or less distinctly defined, often broader than the neck, oblong or ovate in form, rounded or obtuse in front, nearly always with a conspicuous, pale, angular spot on each side. Ocelli numerous, arranged in two frontal clusters on the white marginal area, and in two dorsal groups; each of the anterior or frontal ones consists of numerous small ocelli arranged in two or more close rows forming an oblong or crescent shaped cluster close to the antero-lateral margin of the head; in some cases each of these clusters is double, consisting of a larger, outer or lower group and an upper, smaller one; but these subordinate clusters are usually more or less blended; the dorsal groups are smaller and of fewer ocelli, rounded, and situated at the postero-dorsal part of the head, close to the ganglions, and usually on, or just in front of, a narrow whitish line across the neck which marks the position of the transverse fossæ. Proboscis large, covered with small papillæ. Color of body, above, and middle of head usually deep purple, madder-brown, or purplish brown, sometimes plum-color, chocolate-brown, reddish brown, and orange-brown; sides and lower surface much paler brown, often flesh-color or pinkish. The head is whitish in front and is almost always conspicuously marked with two large angular spots or patches of whitish or flesh-color on the sides above; most frequently these spots are broad, triangular or trapezoidal, with the apices directed toward the median line above, but separated by a wide dorsal stripe of dark color like that of the body; in other cases the apices of these spots are more truncated, giving a broad, somewhat squarish form, the shape varying with the extension of the head; a little back of the spots a narrow angulated white line, corresponding to the transverse fossæ, crosses the neck, but it is sometimes absent; in front of the angular spots there is usually another, more conspicuous, white line or narrow band across the dark pigment of the head, but this is sometimes interrupted dorsally and is then represented by a narrow triangular spot of white on each side of the head; proboscis, when protruded, reddish.

Length up to 100 to 150$^{mm}$; diameter 6 to 8$^{mm}$ or more.

Massachusetts Bay to Gulf of St. Lawrence, Labrador, Cumberland Gulf, and Greenland. Very common and of large size at low-water mark, under stones, at Eastport, Me., and Grand Menan, N. B. I have also dredged it in numerous localities off Nova Scotia; in the Bay of Fundy; off the coast of Maine; Casco Bay; off Cape

Ann; off Cape Cod, etc., in 4 to 150 fathoms; and in the Gulf of St. Lawrence, 15 fathoms.

This large and conspicuous species is generally easily recognized by its clear, dark purplish or chocolate-brown color above, with pale margins and a trapezoidal or triangular white spot on each side of the head, and usually with a narrow white line across the neck; and by the pinkish or flesh-colored lower surface. Ocelli in two or more rows in an elongated group on each antero-lateral margin of the head, and a pair of small sub-dorsal clusters on the transverse white nuchal band.

The *Planaria angulata* of Otho Fabricius was, without doubt, based on this species; but his description being very brief, writers have hesitated in regard to this identification. His description of the characteristic white angular spots on the head, the color, and the habits could, however, apply to no other known species. The rediscovery of this species on the coast of Greenland by Levinsen, and its abundance in Cumberland Gulf, renders it quite certain that Fabricius had this species before him. Hence I have considered it necessary to restore his name.

This species and some of the others herein described, e. g. *A. frontalis*, evidently belong to the group for which Dr. Stimpson instituted the genus *Cosmocephala*. Among the characters given, the clusters of ocelli are said to be arranged on the antero-lateral margins of the head. The cerebral clusters may, perhaps, have been overlooked in at least one species. Dr. Stimpson has described two North Pacific species that are evidently closely allied to *A. angulatus*, viz:

*Amphiporus Beringianus* (*Cosmocephala Beringiana* St.) This was dredged in Bering Straits, in 5 fathoms. It closely resembles a light-colored variety of *A. angulatus* and may be identical with it.

*Amphiporus Japonicus* (*Cosmocephala Japonica* St.) was from Simoda, Japan, low water, among rocks. It differs more from our species than does the preceding. It is brown above, with a pale median line, with irregular pale spots on the head, and triangular cervical spots of white; clusters of ocelli are antero-lateral.*

* Prodromus, in Proc. Phil. Acad. Nat. Sci., ix, p. 165, 1857. The extensive collections of invertebrates made by Dr. Stimpson on the North Pacific Exploring Expedition were nearly all destroyed in the great Chicago fire by which the Museum of the Chicago Academy of Science was burned. His original notes and drawings were burned at the same time. His colored figures of the Turbellaria and Nemerteans, which I had the pleasure of examining not long before the fire, were numerous and excellent. Had he been able to publish his figures subsequent writers would have found it easy to identify his new genera and species, briefly described in the Prodromus.

**Amphiporus multisorus** Verrill, sp. nov.

PLATE XXXIII, FIGURE 3.

Body moderately long, versatile. Head rather wider than the body, rather short, rounded in front, separated from the body by a slightly curved transverse fossa on each side. Front ocelli form six small rounded, submarginal clusters; three clusters each containing 3 or 4 ocelli, are on each side of the front of the head, arranged parallel with the margin; the posterior ocelli form two roundish, subdorsal clusters, each containing 6 to 8 ocelli, situated near the posterior part of the head, just in front of the pink ganglions.

Color of body, salmon or flesh-color, paler beneath.

Length, in extension, 25 to 35$^{mm}$; diameter, 3 to 5$^{mm}$. Described from life.

Eastport, Me., at low water mark, and in 12 fathoms, 1870.

This species, in the form of the body and arrangement of the ocelli, is closely allied to *A. angulatus*, of which I formerly supposed it a pale variety. The very pale colors, total absence of the white patches on the head, and peculiar grouping of the anterior ocelli are characters that seem to warrant its separation as a distinct species, at least until intermediate specimens be discovered.

**Amphiporus heterosorus** Verrill, sp. nov.

*Amphiporus roseus* (*pars*) Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., vol. ii, p. 183, 1879. (*non* Müller.)

PLATE XXXIV, FIGURES 7, 17.

Body rather stout, rounded, obtuse at each end, versatile. Head obtuse, usually rather wider than the body. Ocelli numerous, arranged in a pair of roundish clusters on the posterior part of the head, and in a pair of triangular clusters at the front; these triangular clusters, having their bases at the anterior margin of the head, extend upward and backward to near the middle of the head and end in an acute apex formed by a few ocelli, larger and more distinct than the rest. The posterior groups are smaller, wide apart, and distinctly separated from the anterior ones. A pair of shallow transverse fossæ, on the posterior part of the head, runs upward in line with the posterior groups of ocelli. Anterior fossæ were not noticed. Proboscis clavate in extension, large and long, equal to more than half the length, and about one-half the diameter of the body, finely papillose toward the end, and light brownish red in color.

Color of body, above, cherry-red, clear reddish brown, or light chocolate-brown; the sides and ventral surface flesh-color; a dark, medial, longitudinal line on the head.

Length, in extension, 30$^{mm}$ to 50$^{mm}$; diameter 3$^{mm}$ to 5$^{mm}$; length of proboscis, in extension, 25$^{mm}$; diameter 1·5$^{mm}$ (No. 5).

The specimens described above, from life, were taken off Cape Ann, Stat. 136 (U. S. F. C.), in 26 fath., sand, 1878. Eastport, Me.; Bay of Fundy; Gulf of Maine; Casco Bay; Massachusetts Bay; off Cape Cod; common in 10 to 200 fathoms, on muddy and sandy bottoms.

## Amphiporus tetrasorus Verrill, sp. nov.

PLATE XXXIV, FIGURE 6.

Body very changeable, in extension roundish, rather thick, tapering but little, obtuse at both ends. Head as wide as body, usually obtuse or subtruncated in front, separated from the body by conspicuous transverse fossæ which curve upward and forward on each side; on the under side of the head these fossæ run forward, on each side, to the mouth. Ocelli numerous, forming two oblique, oblong, nearly parallel clusters on each side, the posterior ones just in front of, and parallel with, the transverse fossæ.

Color of body, above, chocolate-brown, darker medially; head, in front of eyes, white; body, beneath, whitish.

Length, 25 to 30$^{mm}$; diameter, 2$^{mm}$. The specimen described above, from life, was dredged at Station 132 (U. S. Fish Com.), off Cape Ann, Mass., in 45 fathoms, mud, July, 1878.

## Amphiporus lactifloreus (Johnston) McIntosh.

*Planaria lactiflorea* Johnston, Zool. Journal, vol. iii, p. 489, 1828.

*Nemertes lactiflorea* Johnston, Mag. Zool. and Bot., vol. i, p. 535, pl. XVII, f. 2 and 3, 1837.

*Borlasia alba* W. Thompson, Ann. Nat. Hist., vol. xv, p. 320 (with woodcut), 1845.

*Polia mandilla* Quatrefages, Ann. des sc. nat., 3$^{me}$ sér., Zool., tom. vi, p. 203, tab. 8, figs. 1 and 1*a*, and tab. 9, fig. 2, 1846.

*Nemertes mandilla* Diesing, Syst. Helm., vol. i, p. 274, 1850.

*Omatoplea mutabilis* Diesing, op. cit., p. 262, 1850.

*Omatoplea rosea* Johnston, Catalogue Brit. Mus., p. 23, plate II*a*, f. 2, 2*, 2**, 3, and 3*, 1865.

*Omatoplea alba* Johnston, op. cit., p. 23, 1865.

*Amphiporus lactifloreus* McIntosh, British Annelids, Part I, Nemerteans, p. 156, plate 1, figs. 1 and 2, 1873; Jensen, Turbellaria ad Lit. Norvegiæ, p. 80, 1878.

*Amphiporus lactifloreus* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 184, 1879.

PLATE XXXIX, FIGURES 7, 7*a*.

Body rather elongated, roundish above and on the margins, flattened beneath, of nearly uniform breadth from the head to near

the posterior end. Head often somewhat expanded, a little flattened, obtuse or subacute in front according to state of extension. Ocelli form, on each side of head, a nearly simple submarginal row on the antero-lateral part, and behind the ends of each of these rows there is a small cluster of about three or four ocelli on each side, near the ganglions.

Color dull white, grayish, or pale flesh-color, often with a darker stripe along the back due to the proboscis-sheath; along the margins, especially beneath, the lateral sacs of the alimentary canal are often visible. Length 50 to 75$^{mm}$; diameter 4 to 6$^{mm}$, in extension. Eastport, Me., and Grand Menan, N. B., at low-water mark, under stones.

This species, which is here referred, with some doubt, to the European form, is not uncommon on the shores of the Bay of Fundy.

## Amphiporus roseus (Müller).

*Fasciola rosea* O. F. Müller, Verm. terrest. et fluv. hist., i, 2, p. 56, 1774.

*Planaria rosea* Müller, Zool. Danic. Prodr., p. 221, No. 2679, 1776; Zool. Danic., vol. ii, p. 31, tab. 64, fig. 1 and 2, 1788.

*Nemertes pulchra* Johnston, Mag. Zool. and Bot., vol. i, p. 536, pl. XVII, fig. 6, 1837.

*Polystemma roseum* Œrsted, Kroyer's Nat. Tidss., vol. iv, p. 579, 1837.

*Polystemma pulchrum* Œrsted, op. cit., p. 580, 1837.

*Omatoplea rosea* (*pars*) Diesing, Syst. Helm., vol. i, p. 251, 1850.

*Omatoplea pulchra* Diesing, op. cit., p. 252, 1850.

*Ommatoplea pulchra* Johnston, Catalogue Brit. Mus., p. 24, pl. IIa, fig. 6 and 6*, 1865.

*Amphiporus pulcher* McInt., British Annelids, Part I, Nemerteans, p. 158, pl. I, fig. 3; PL. XIV, fig. 11, 1873.

PLATE XXXIV, FIGURES 5, 5*a*, 5*b*.

Body rather stout, not much elongated, tapering somewhat to both ends. Head usually broader than the body, ovate in extension, obtuse in front, separated from the body by a slightly marked, curved, transverse groove or fossa on each side. Ocelli numerous, arranged somewhat in four groups, the anterior pair lateral or submarginal, the posterior subdorsal; the anterior clusters form long, crescent-shaped groups or nearly simple rows on each side, running somewhat parallel with the antero-lateral margins of the head, but curving inward posteriorly, so that their posterior ends nearly meet on the median dorsal surface; the two posterior groups, which are opposite the hinder portion of the crescents and nearer the postero-lateral margins of the head, have an irregular roundish or ovate

form and are often almost united to the front groups by a few ocelli scattered between them.

Color of body, above, clear orange-red, paler beneath.

Length of the specimen described above, from life, 18 to 20$^{mm}$; breadth 5$^{mm}$. Massachusetts Bay to Bay of Fundy, in various localities, low-water to 112 fathoms. The specimens above described were taken at station 38, 1877, in 112 fathoms, off Grand Menan.

**Amphiporus ochraceus** Verrill, Check List Invert., 1879.

*Cosmocephala ochracea* Verrill, Invert. of Vineyard Sound, etc., pp. 31, 336, pl. XIX, figs. 95, 95*a*, 1873.

PLATE XXXIII, FIGURES 5, 6; PLATE XXXIX, FIGURE 8.

Body elongated, moderately slender, somewhat flattened, but thick, with the margins rounded, obtuse at both ends, or subacute posteriorly; broadest and often swollen anteriorly; gradually and

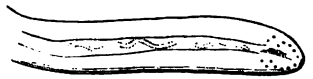

Fig. 2. *Amphiporus ochraceus.* Head and part of body, to show ocelli, enlarged.

slightly tapering posteriorly; the integument is translucent and the internal organs show quite distinctly; lateral (saccular) organs voluminous, extending nearly the whole length of the body along each side, and showing through as dull yellowish white mottlings. Head usually ovate, slightly wider than the body, obtuse; a slight fossa or groove, usually appearing as a whitish line on each side, runs obliquely across the ventral and lateral surface of the head, diverging from the mouth and curving somewhat forward and upward at the sides; another, less distinct, is situated farther forward on each side of the head. Proboscis-pore small and inconspicuous in contraction; mouth, small. Ocelli numerous, but varying somewhat in number; the anterior ones form a submarginal curved row along each side of the head, anteriorly, but curve inward farther back; just back of these, on each side, there are usually four distinct posterior ocelli, standing two by two, obliquely. Color dull yellowish, or yellowish white, often tinged with deeper yellow or orange anteriorly, with the median line lighter; the position of the cephalic ganglions is shown by faint reddish spots between the posterior groups of ocelli.

Length, 50$^{mm}$ to 70$^{mm}$; breadth, 2·5$^{mm}$ to 3$^{mm}$.

The proboscis is large and thick (Pl. XXXIX, fig. 8). The central stylet (Pl. XXXIII, fig. 5*a*) has a rather narrow, oblong shaft, rounded at the base, and with broad basal alæ; the two lateral sacs usually contain only two stylets each.

Common between tides and in tide pools, under stones and creeping among algæ, hydroids, bryozoa, etc., on the piles of wharves and other similar places. Also dredged frequently in 2 to 20 fathoms, on stony or shelly bottoms, off New Haven, Conn.; Thimble Islands; Noank, Conn.; Newport, R. I.; Woods Holl, Mass.; also dredged at numerous other localities in Long Island Sound and Vineyard Sound. North of Cape Cod it is less abundant, but I have dredged it at many stations, at moderate depths, in Massachusetts Bay. It also occurred between tides on the north shore of Cape Cod, at Provincetown and Barnstable, Mass.

## Amphiporus glutinosus Verrill.

*Polina glutinosa* Verrill, Invert. Animals of Vineyard Sound, etc., p. 337, plate xix, fig. 97, 1873.

PLATE XXXV, FIGURE 5.

Body rather slender and elongated in extension, usually broadest in the middle and tapering to both ends, but quite versatile in form; head not distinct, usually obtuse; posterior end narrower, usually obtuse or slightly emarginate; integument soft, secreting a large quantity of mucus; the lateral organs extend close to the head. Ocelli numerous, variable in number, usually eight or ten on each

Fig. 3. *Amphiporus glutinosus.* Outline, enlarged.

side, arranged in three pairs of short, oblique, divergent rows, two to four in each; proboscis-pore moderately large terminal; no lateral fossæ were observed. Color dull yellow or pale orange-yellow, sometimes brighter orange, especially anteriorly; posteriorly usually lighter, with a faintly marked dusky or greenish median line.

Length, $25^{mm}$ to $30^{mm}$ in extension; breadth, $1{\cdot}3^{mm}$ to $2^{mm}$. Great Egg Harbor, N. J., to New Haven, Conn., and Wood's Holl, Mass.; low-water mark to 6 fathoms, usually among hydroids and bryozoa.

**Amphiporus griseus** (Stimp.) Verrill.

*Polina grisea* Stimp., Prodromus, in Proc. Philad. Acad. Nat. Sci., vol. ix, p. 164, 1857.

Body rather long, a little depressed, sub-cylindrical in extension, pale gray in color. Head distinct, ovate, or subcordate, narrower than the body, acute in front. Anterior clusters of ocelli larger, elongated, partly submarginal on the antero-lateral margin of the head; ocelli ten in each cluster. Posterior clusters cervical, small, linear, with four ocelli in each.

Length 0·8 inch; breadth 0·04 inch.

In the harbor of Norfolk, Va., sublittoral, among algæ in muddy places.

The above is a translation of Dr. Stimpson's Latin diagnosis. The species appears to be closely allied to *A. glutinosus*.

**Amphiporus frontalis** Verrill, sp. nov.

PLATE XXXIV, FIGURES 1, 1*a*, 1*b*, 8.

Body large, versatile in form, rather elongated, convex above, but somewhat depressed in extension, of nearly uniform breadth to near the ends, which are obtuse. Head in extension usually broader than the neck and separated by a slight constriction, usually longer than broad, but it may shorten into short ovate or broad rounded forms; front margin often emarginate. A well-marked, but shallow, oblique, transverse, ciliated fossa at the posterior border of the head, on each side, curves inward and usually somewhat forward, but does not reach the middle line; in some states of contraction these fossæ curve backward; underneath, the fossæ run very obliquely backward and inward, when the head is extended. Near the front of the head, on each side, a short curved fossa runs inward and curves forward, nearly parallel with the posterior ones, beneath the head they curve inward and backward but they recede in a V-shaped curve on each side of the head. Ocelli rather large and conspicuous, blackish, arranged in a single irregular cluster, or double row, of six to eight or more, on each side of the front and near the margin of the head. Mouth close to the proboscis-pore.

Color translucent white, or pale gray, or yellowish, with a darker dorsal band; sides of body mottled with pale pink or yellowish, due to the internal organs.

A variety taken at Eastport, Me., at low water, 1868, was translucent pale salmon, or flesh-color, mottled laterally with purplish and yellowish, due to the internal organs, while the median dorsal

region was greenish, apparently due to the contents of the intestine. Ocelli about 10 in each cluster.

Length, in extension, 100$^{mm}$; diameter 3$^{mm}$.

Length 25 to 125$^{mm}$; breadth about 3 to 5$^{mm}$. An individual 5 inches long can contract to less than 2 inches.

Eastport, Maine, low water, 1868 and 1870.

**Amphiporus mesosorus** Verrill, sp. nov.

PLATE XXXIV, FIGURE 9.

Body not much elongated, rather thick, well-rounded. Head of the same breadth as the neck, obtuse in front. Posterior transverse fossæ rather shallow and indistinct. Ocelli numerous, forming a large, irregular, somewhat triangular cluster on each side of the middle of the head, the apex of the groups pointing backward toward the ganglions. In some cases these clusters seem to consist of two rather roundish cerebral groups, which blend with two short, triangular lateral groups.

Color above, bright red; beneath, flesh-color. Length 50$^{mm}$; breadth 3$^{mm}$.

Massachusetts Bay, off Salem, Aug. 13, 1877, station 30 (three specimens).

**Amphiporus cruentatus** Verrill, Proc. U. S. Nat. Mus., vol. ii, p. 184, 1879.

PLATE XXXIII, FIGURES 7, 8, 8*a*; PLATE XXXV, FIGURE 3; PLATE XXXIX, FIGURE 9.

A species peculiarly characterized by having red blood, so that the longitudinal vessels appear distinctly red through the translucent integument. Body soft, flaccid, versatile, in full extension slender, tapering to both ends, but capable of becoming thicker and obtuse or even swollen posteriorly, and of contracting into a short stout form. Head not very distinct, scarcely broader than the neck, snout strongly ciliated. Ocelli about 8 to 12 on each side of the head, in a simple, interrupted, longitudinal, sublateral row, the most anterior ocellus distinctly the largest. Two slight transverse grooves on each side of the head, apparently not extending across the dorsal side, the anterior ones curving forward in front of, and the posterior ones behind the ganglia. Proboscis long, densely covered with elongated, conical papillæ; a simple central stylet, with two small, pin-shaped lateral ones on each side (Pl. XXXIX, fig. 9).

Color light flesh-color or yellowish white with conspicuous bright red median and lateral blood-vessels.

Length $25^{mm}$ to $40^{mm}$.

Vineyard Sound, 4 to 10 fathoms; off Newport, R. I., 3 to 8 fathoms. Not very common.

**Amphiporus virescens** Verrill, Proc. Nat. Mus., p. 183, 1879.

*Nemertes*, sp. undet. (*c*) Verrill, Invert. Vineyard Sd., p. 335, 1873.

PLATE XXXIII, FIGURES 4, 4*a*, 4*b*, 4*c*, 4*d*, 4*e*.

Body, in extension, broadest anteriorly, rather depressed, long, slender, tapering gradually to the rather attenuated posterior end. Active in its movements. Head changeable in form, rather large, in expansion usually ovate, broader than the body, depressed, and obtusely rounded in front. A pair of faintly marked, nearly transverse fossæ runs up on each side of the posterior part of the head, crossing the rows of ocelli; farther forward and parallel with these there is another pair of similar furrows that cross the eye-patches, and beneath the head curve forward to the mouth. Ocelli numerous, forming a long lateral cluster on each side of the head; anteriorly each cluster consists of three or more rows, but backward the interior rows cease, finally leaving only the outer row, which extends back beyond the head and neck. Proboscis in partial extension clavate and covered with prominent papillæ; central stylet with an oblong shank, which in one mounted specimen is light greenish blue, together with the transverse, pigmented band near it.

Color clear light green, varying in tint.

Length of largest specimen seen, about $40^{mm}$. New Haven and Noank, Conn.; Newport, R. I.; Wood's Holl, Mass., etc. Common in shallow water among hydroids and ascidians, and on the piles of wharves between tides.

**Amphiporus agilis** Verrill.

*Ophionemertes agilis* Verrill. Am. Jour. Science, vii, p. 45, pl. 7, fig. 1, 1873; Verrill, Expl. of Casco Bay, in Proc. Amer. Assoc. for 1873, p. 389, pl. 2, fig. 4.

*Amphiporus agilis* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 183, 1879.

PLATE XXXV, FIGURE 4.

Body versatile, slender and elongated in extension, slightly depressed, with the sides well rounded, thickest in the middle, tapering gradually to the slender, obtuse posterior end. Head somewhat separate from, and wider than, the anterior part of the body, changeable in form, often oval, sometimes sub-triangular, generally

longer than broad, narrowed anteriorly, obtuse or slightly emarginate, with a terminal proboscis-pore. Ocelli numerous, forming

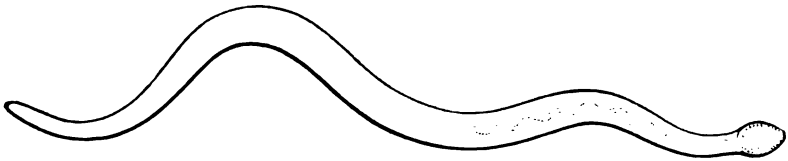

Fig. 4. *Amphiporus agilis.* Outline, enlarged.

a long, crowded, lateral row or group along each side of the head; the rows are simple and convergent anteriorly, posteriorly they become broader and double. Back of the ocelli there is a curved transverse groove or fossa, crossing the back of the head. No anterior fossæ were observed. Color pale ocher-yellow; median dorsal line slightly reddish; the internal lateral organs lighter yellow, giving a reticulated appearance to the sides.

Length $25^{mm}$ to $40^{mm}$; diameter $1{\cdot}5^{mm}$ to $2^{mm}$. Described from life (No. 546).

Casco Bay, 20 to 65 fathoms; Bay of Fundy, 10 to 90 fathoms; Massachusetts Bay and off Cape Cod, 12 to 60 fathoms.

This species is very active and restless. It creeps with a rapid gliding motion, frequently moving its head from side to side, and in confinement is apt to creep above the edge of the water and perish by drying up. It secretes mucus abundantly and forms tubes of that material. It also creeps on the surface of the water, back downward, like most of the species of *Tetrastemma*, which it closely resembles in habits.

## Amphiporus bioculatus? McIntosh.

PLATE XXXIV, FIGURES 3, 4, 15.

Body rarely more than 1·5 inches long, soft, changeable in form, in extension usually rather short and thick, roundish, tapering only slightly toward the ends, which are usually obtuse; the posterior region is sometimes broader; head not wider than the body, not distinctly defined, in extension tapering to the front end, which is usually subacute; ocelli two, forming a pair, close together and near the front margin of the head. A pair of small, rather faint, anterior transverse fossæ passes upward and forward on the sides of the head, just back of the eyes, usually showing only as pale lines, apparently not meeting dorsally.

Color dark orange-red, varying to pale orange and salmon, with paler margins and ventral surfaces and usually with darker brownish mottlings along the sides posteriorly, due to the internal organs;

the large proboscis-sheath and proboscis can often be seen indistinctly along the median dorsal region.

Length, in extension, about 35 to 40$^{mm}$; diameter 2·5 to 3·5$^{mm}$.

Length of a specimen from station 811, in 19 fathoms, 18 to 20$^{mm}$; diameter 1·25 to 2$^{mm}$.

Proboscis large; its armature consists of a slender central stylet having an elongated, narrow, oblong or cylindrical shaft, surrounded by a small dark-colored basal expansion. Below the stylet there is a dark pigmented transverse band. Described from living specimens.

Long Island Sound, near New Haven, etc.; Fisher's Island Sound; and Vineyard Sound, in 1 to 10 fathoms, not uncommon. Noank, Conn., in harbor mud, 1874. Off Block I., 19 fath., sand, (Sta. 811).

### Amphiporus cæcus Verrill, sp. nov.

*Nemertes?*, sp. undet. (*a*), Invert. of Vineyard Sound, etc., p. 335, 1873.

PLATE XXXIV, FIGURES 2, 2*a*, 2*b*, 2*c*.

Body soft, oblong, flattened, obtuse at both ends, the edges rounded. Head not distinctly separated from the body and of the same breadth; a faint whitish groove crosses the neck, receding in the middle above, and extends around on each side to the ventral surface, on which it advances in the middle, or runs directly across, according to the state of contraction. No ocelli. The cephalic ganglions can usually be seen through the integument of the head, especially on the lower side, as reddish spots.

Color bright orange-red; lighter orange-yellow along the sides; usually with a median dorsal stripe of darker red.

Length, in extension, 35 to 40$^{mm}$; diameter 2·5 to 3$^{mm}$. Described from living specimens.

North of Block Island, 18 to 20 fathoms, Aug. 6, 1874.

### Amphiporus cæcus Young.

Body very slender. Head acute. Ocelli none. Proboscis with a central stylet having a narrow oblong shaft and expanded base, much as in that of *A. ochraceus;* lateral stylets not observed, perhaps wanting.

Color pale yellowish white, with the head red.

Length about 6 to 7$^{mm}$.

Station 812, in 28 fathoms, sand. Off Block Island, 1880.

**Amphiporus thallius** Verrill.

*Amphiporus* sp., Verrill, Bulletin U. S. Nat. Mus., No. 15, p. 143, 1879 (with description).

Body, as preserved in alcohol, thick, not very long, somewhat depressed, tapered a little to both ends, which are obtuse. Head not very distinct, of the same width as the body; transverse fossæ at back part of head not very distinct, running back obliquely on each side, so as to form a V-shaped line on the middle above. Ocelli minute, arranged in a small roundish cluster on each side, on the pale antero-lateral margins.

Color, in alcohol, dark bluish green above; under surface and margins of head yellowish white. In life "bright pea-green" (Kumlin). Length, in alcohol, 25 to 30$^{mm}$; diameter 4 to 5$^{mm}$.

Cumberland Gulf, N. lat. 66°, October 4, 1887; Arctic Island, at low water, Sept. 13, 1877 (Kumlin coll.).

The very peculiar and strongly marked color, which persists for years in alcoholic specimens, appears to be characteristic of this species.

**Amphiporus (?) superbus** Verrill.

*Nareda superba* Girard, in Stimpson, Invert. Grand Menan, p. 28, pl. 2, fig. 17, 1853.

PLATE XXXIV, FIGURE 16.

This species was dredged off Grand Menan in 40 fathoms by Dr. Wm. Stimpson. The description by Girard was evidently based on the drawing furnished him by Dr. Stimpson, and could not have furnished anything more than the external appearance. I have reproduced the original figure, somewhat reduced by photography. The original description is as follows:

"NAREDA Grd."

"Body elongated, subcylindrical. Head obtusely triangular in front, neck slightly contracted; one pair of rounded ocelli."

"*N. superba* Grd.—Length from one to two inches; body posteriorly attenuated; head forming an equilateral triangle; the base of which is at the contracted neck. Color above uniform soft red; head margined by a narrow band of white. The neck is also marked by a transverse band of white, on which the eyes are situated, far apart. Below white. Dredged in thirty-five fathoms, in the Hake Bay."

The only character mentioned which could have been considered as of generic value is the presence of two eyes (?) on the white

nuchal band. But it is much more probable that those spots represented either the cephalic ganglions, which usually show themselves in that position in this group, or else clusters of small ocelli.

In the latter case there may also have been small anterior ocelli that were overlooked. Either supposition is consistent with its supposed relation to the known species of *Amphiporus* with many of which it agrees in form.

Although I have spent part of several summers dredging in the region of Grand Menan, and have dredged even in the same locality where this species was obtained, I have never met with a Nemertean that could be referred to the same species with certainty, even after making allowances for errors in the original drawing. The nearest approach to it that I have seen, is a red variety of *A. angulatus* in which the angular pale spots on the sides of the head are nearly obsolete, and the front ocelli inconspicuous. I have, therefore, reproduced the original description and figure.

## Tetrastemma Ehrenberg, 1831.

*Polia* (*pars*) Quatr, Ann. des sci. nat., vol. vi, 1846.

*Œrstedia* Quatr., op. cit, p. 221; Dies., Syst. Helm., vol. i, p. 247 (*non* Hubrecht).

*Tetrastemma* Diesing, Syst. Helm., vol. i, p. 256, 1850; Stimpson, Prodromus, in Acad. Nat. Sci. Philad, p. 163 [19], 1853.

*Nemertes* (*pars*) Dies., Syst. Helm., vol. i, p. 269, 1850.

Body rather small, moderately elongated, often nearly terete. Head in some species wider than neck, but in many species of the same breadth. Transverse fossæ usually two on each side of the head, more or less oblique. Ocelli four, arranged in a quadrangle. Proboscis with a central stylet and two lateral chambers, each usually containing two to four stylets.

A terrestrial species of *Tetrastemma* (*T. agricola*) has been described from the Bermudas by Moseley. Fresh water species of the same, or a closely allied genus, are also known.

## Tetrastemma candidum (Fabr.) Œrsted.

? *Fasciola candida* O. Fabr. in O. F. Müller, Verm. terrest. et fluv. hist., I, ii, p. 71, 1774.

? *Planaria candida* O. Fabr. in O. F. Müller, Zool. Dan. Prodr., p. 223, No. 2704, 1776; O. Fabricius, Fauna Grœnlandica, p. 327, 1780.

*Planaria quadrioculata* (*pars*) Johnston, Zool. Jour., vol. iv, p. 56, 1829.

*Nemertes quadrioculata* Johnston, Mag. Zool. and Bot., vol. i, p. 535, pl. XVII, fig. 4. 1837.

*Tetrastemma varicolor* (*pars*) Œrsted, Kroyer's Naturhist. Tidss., iv, p. 575, 1837; Diesing, Syst. Helm., vol. i, p. 257, 1850.

*Tetrastemma grœnlandicum* Diesing, op. cit., p. 259.

*Tetrastemma candida* McIntosh, British Annelids, Part I, Nemerteans, p: 167, pl. II, figs. 2, 3, 1873 (*non* Diesing, Syst.); Levinsen, Grœnlands Turbell., p. 39 [200], 1879; Verrill, Amer. Jour. Sci, vol x, p. 40, 1875; Check List, 1879.

PLATE XXXIII, FIGURES 9, 10, 10*a*; PLATE XXXV, FIGURES 9, 10.

Body very contractile, in extension slender, elongated, somewhat depressed, tapering backward and often attenuated toward the posterior end. Head in usual extension rather wider than the body. Ocelli rather large, conspicuous, reddish brown, nearly in a square, but when the head is fully extended, the two pairs are farther apart than the distance between those of a pair.

Color variable, usually pale green, greenish white, or yellowish white, translucent, and generally with indistinct lateral grayish mottling, due to the internal organs; sometimes the intestinal area is decidedly greenish, while the sides are pale yellow; at other times the median region is whitish and the sides pale green. Several specimens, taken at Eastport, Me., in South Bay, 8 to 10 fathoms, mud, 1868, were clear cream color above, whitish below.

Length in extension 25 to 32$^{mm}$; diameter 1 to 2$^{mm}$.

Common at many localities between tides, among algæ, hydroids, and bryozoa from New Haven, Conn., to the Bay of Fundy. Also dredged at moderate depths, 1 to 14 fathoms, in many localities.

This species is very active; it creeps rapidly with a gliding motion. The relatively larger size of the head, more conspicuous eyes, and lighter colors, as contrasted with the following species, are its most distinctive characters.

It seems to me very doubtful whether the *Planaria candida* of Fabricius was this species. The large size and the habits given by him, and lack of mention of the eyes are against that view. His species may have been *Amphiporus lactifloreus*, a Greenland species.

## Tetrastemma candida. Variations.

Several specimens of the variety figured on plate XXXIII, figs. 10, 10*a*, were taken on the piles of the wharves at Gloucester, Mass., July 24, 1878. These were probably not full grown. The body was 8 to 12$^{mm}$ long, in extension, slender, very changeable, usually of nearly uniform breadth to near the ends. Head obtuse and usually a little wider than the body, but very changeable in shape; when extended the ocelli were farther apart longitudinally than transversely, but when the head contracted, as in progression, the two pairs of ocelli were brought near together, as shown in the

figures. The form and direction of the two pairs of rather indistinct, transverse cephalic fossæ also varied greatly with the changes in the shape of the head. The two lateral stylet-sacs of the proboscis contained three stylets each.

Color of body, above, pale yellowish green, or pale brown; head with an opaque, flake-white spot in front of the eyes; along the margins of the body the internal organs produce series of brownish, irregular, transverse spots or blotches, varying in depth of color; alternating with these spots, and so interrupting the marginal dark bands, there are small, rounded whitish spots, probably due to the ovaries.

### Tetrastemma elegans Verrill.

*Tetrastemma elegans* Verrill, Amer. Journ. Sci., vol. x, p. 40, 1875.
? *Hecate elegans* Girard, Proc. Boston Soc. Nat. Hist., vol, iv, p. 186, 1852.

PLATE XXXIV, FIGURE 10.

Body, in extension, longer and more slender than most species of the genus, depressed, broadest in the middle, tapering both ways. Head ovate, broader than the neck, obtuse or emarginate in front; lateral fossæ not very distinct. Ocelli conspicuous, nearly in a

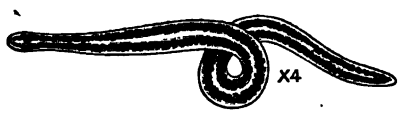


Fig. 5. *Tetrastemma elegans.* Dorsal view.

square, the front pair rather nearer together than the others. Color above, striped with two broad brown lateral, and a wide median, yellow stripe; the median stripe is clearly defined, clear light yellow and occupies about a fourth of the breadth of the back; it extends to the front of the head, becoming narrow on the neck and then expanding on the middle of the head; a narrow ring of light yellow surrounds the neck, just behind the head; the two stripes of dark brown are well defined, but have irregular margins and are varied in color by paler specks; lower surface and margins of body and head pale yellow.

Length $20^{mm}$; breadth 1 to $1{\cdot}5^{mm}$. Described from life.

Noank, Conn., among eel-grass; Fisher's Island Sound, 2 to 8 fathoms; Wood's Holl, Mass., on piles of wharf.

A paler variety occurs in which the lateral bands are lighter brown, interrupted by yellowish spots, and the dorsal stripe is less clearly defined.

**Tetrastemma vermiculus** (Quatr.) Stimpson.

*Polia vermiculus* Quatrefages, Ann. des sc. nat., ser. III, Zool., vol. vi, p. 214, 1846; Voyage en Sicilie, vol. ii, p. 126, pl. XIV, figs. 12, 13, 1849.

*Nemertes vermiculus* Diesing, Syst. Helm., vol. i, p. 270, 1850.

*Tetrastemma vermiculus* Stimpson, Proc. Acad. Nat. Sci. Philad, vol. ix, p. 163 (19), 1857; Diesing, Revis der Turbell., p. 290, 1862.

*Tetrastemma vermicula* McIntosh, British Annelids, Part I, Nemerteans, p. 169, Plate III, fig. 3, 1873.

*Tetrastemma vermiculus* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 184, 1879; Check List, 1879.

PLATE XXXIII, FIGURES 11 to 11*c*; PLATE XXXIV, FIGURES 11, 12; PLATE XXXV, FIGURES 8, 11.

Body versatile, rather slender in extension, obtuse at both ends; sometimes tapered to the posterior end, but more often of nearly uniform diameter. Ocelli conspicuous, the two pairs rather far apart when the head is extended, those on the same side farther separated than those of a single pair, and connected by a dark line of pigment, which is rarely absent.

Color rather variable; above, often pale yellowish, or pale salmon, or translucent yellowish gray, more or less specked or spotted, especially along the sides, with brown, often leaving a paler, wide, rather indefinite dorsal stripe; ventral surface and front of head pale.

Length, in extension, about $20^{mm}$; breadth $1^{mm}$. Described from life.

Many specimens taken at Wood's Holl in the mud of Little Harbor, July 25th, 1881, and August 4th, 1882, varied from dull orange-yellow to bright greenish yellow, more or less covered with specks of brown, especially on the sides, yet not forming a definite dorsal stripe, but with a darker brownish, often indistinct stripe on each side of the head between the eyes.

Length up to 18 to $20^{mm}$.

*Young*—several young specimens of this species were taken together in a tide-pool, in 1878 (No. 12).

Body slender, of nearly the same width throughout. Ocelli conspicuous, the two pairs more widely separated than usual when the head is extended; the front ones a little larger than the others. Color translucent pale yellow, bright salmon, and flesh-color, usually with a white median spot in front of the anterior ocelli, and sometimes, also, with other white specks along the back; frequently an irregular brownish band runs along each side of the back; median line paler. In many of the specimens a faint longitudinal line of

dark brown pigment specks runs between the ocelli on each side. In one example the median region, posteriorly, was green probably from the contents of the intestine showing through the integument.

Gloucester, Mass., at Ten Pound Island, in a tide-pool at low-water, among algæ.

This European species is common among hydroids, bryozoa, ascidians, etc., between tides, on rocks, piles of wharves, and in tide-pools, from Long Island Sound to the Bay of Fundy. I have, also, often dredged it in 2 to 12 fathoms, at various localities on hard bottoms. It is especially abundant among ascidians in Vineyard Sound, in 6 to 10 fathoms. Very common at Noank, Conn., in the harbor, on muddy bottoms among eel-grass. Common in similar places and on piles of wharves, at Wood's Holl, Mass., and Newport, R. I.

### Tetrastemma vermiculus, variety catenulatum nov.

PLATE XXXIV, FIGURE 12; PLATE XXXV, FIGURE 11.

Form and size essentially as above described. Ground-color, above, light salmon, pale yellow, or yellowish gray, thickly covered along the sides with irregular specks, spots, or blotches of brown, which at more or less irregular intervals extend upward toward or across the middle line, interrupting the median dorsal light stripe, which is often thus divided into a series of irregular oblong or elliptical spots; sometimes there is also a row of small brown spots along the median line; middle of head pale, often with flake-white specks; stripe of dark color, more or less distinct, between the two eyes of each side; lower surface pale yellow or yellowish white. Length up to $18^{mm}$; diameter $1^{mm}$. Described from life. Specimens of this marked variety are common in the harbor at Wood's Holl, Mass.

Other specimens, from the same locality, were noted as follows:

Body very changeable, often, in extension, narrow or sub-acute at both ends and more or less swollen in the middle, at one or more places, at other times nearly cylindrical or terete. Color pale yellowish or grayish green, with a darker central line on the pale dorsal stripe and with irregular, transverse, lateral markings. Or specks of darker brown are scattered over the back, and are often arranged in imperfect lateral stripes, leaving a paler, wide, more or less irregular and interrupted median stripe; lower surface pale. Ocelli reddish brown, forming nearly a square. Usually a line of dark pigment connects the two ocelli of the same side (No. 857).

Tetrastemma dorsale (Abildgaard) McInt.

*Planaria dorsalis* Abildgaard, Zool. Danic., vol. iv, p. 25, tab. 142, figs. 1–3, 1806.
*Tetrastemma fuscum* Œrsted, Kroyer's Naturhist. Tidss., iv, p. 575, 1844.
*Œrstedia maculata* Quatr., Ann. Sci. Nat., ser. III, vol. vi, p. 222, pl. VIII, fig. 2.
*Tetrastemma marmoratum* Claparède, Beobach. über Anat. u. Entwicklung., etc., p. 24, pl. v, fig. 14, 1863 (variety).
*Tetrastemma variegatum* Johnston, Catalogue Brit. Mus, pp. 20 and 289, 1865.
*Tetrastemma dorsalis* McIntosh, British Annelids, Part I, Nemerteans, p. 172. pl. I, fig. 4; pl. III, fig. 4, 1873; Verrill, Check List, 1879.
*Tetrastemma dorsale* Jensen, Turb. ad Litoria Norvegiæ, p. 81, pl. VIII, figs. 9 to 12, 1878.

PLATE XXXIV, FIGURES 13, 14.

Body only moderately elongated, sub-terete, usually nearly cylindrical in extension, with both ends obtuse. Head not wider than the body, with two rather indistinct transverse fossæ on each side. Ocelli forming nearly a square; in full extension more distant longitudinally than transversely. Proboscis-pore a little below the end of the snout. Proboscis large, when protruded more than three-fourths the length of the body, thickly covered with acute papillæ.

Color variable; generally brown or dull reddish, with a well defined light dorsal stripe; or else variegated or mottled with two or more shades of brown, with or without the dorsal stripe.

Length up to $20^{mm}$; diameter 1·5 to $2^{mm}$.

*Variations.*—Among the variations noted in life, are the following:—

Several examples were taken together at stations 310 to 313, off Cape Cod, in 15 to 21 fathoms, 1879.

In these the general color above was brownish, with a conspicuous pale, flesh-colored dorsal stripe, bordered with dark brown on each side; the brown lateral stripes were freckled with white specks; a pale line crosses the neck behind the eyes; front of the head, margins of the body and tip of the tail pale flesh-color. Pl. XXXIV, fig. 13.

Length 12 to $18^{mm}$; diameter 1·5 to $2^{mm}$.

Other specimens had the following characters:

Body slender, $10^{mm}$ long; $1^{mm}$ broad. Color cinnamon-brown, specked with darker brown, and with a pale median line. Ocelli conspicuous, black. When the head is extended the two pairs are more distant than the space between those of the same pair. Proboscis large, more than three-fourths as long as the body, thickly covered with acute, conical papillæ, and protruded from a large pore, which is sub-ventral. Two slight transverse fossæ are seen on each side of the head.

Broad Sound, Casco Bay, July 22, 1873 (No. 72*i*).

**Tetrastemma dorsale**, variety **marmoratum** (Clap).

PLATE XXXIV, FIGURE 14.

Body terete, somewhat elongated in extension, obtuse at both ends. Proboscis large, protruded from the sub-terminal pore, thickly covered with papillæ. Color pale olive-brown, or chestnut-brown, irregularly mottled and blotched with darker brown.

Length $18^{mm}$; diameter $1{\cdot}5^{mm}$. Described from life (No. 735).

Portland, Me., in 2 to 3 fathoms, harbor mud, July 28, 1873.

A paler colored race, probably closely related to this variety, was taken in the harbor of Eastport, Me., in 12 fathoms, in 1872 (No. 507). The body was changeable in form, usually nearly cylindrical, and obtuse at both ends. Ground-color pale yellow or salmon, thickly blotched and mottled with dark brown, or greenish brown; some specimens had an inconspicuous ring of yellowish white around the neck. Length 15 to $18^{mm}$; diameter 1·25 to $2^{mm}$.

**Tetrastemma dorsale**, variety **unicolor** Verrill, nov.

A specimen taken in Eastport Harbor, off Friar's Head, in 18 fathoms, August 20, 1870, agreed, with this species in form but differed so much in color that it probably ought to be considered as representing a distinct variety, at least:—

Body moderately slender, slightly depressed, with the sides rounded. Head obtuse, four distinct black ocelli. Color, above, uniform dark fuscous brown; lower surface paler.

Another specimen taken in 1879, according to the notes made from life, probably belongs to the same variety:—

Body moderate in extension, broadest at or behind the middle, tapering to both ends, not very slender. Ocelli well developed, the two pairs, when the head is extended, wider apart than the distance between those of the same pair. A very distinct transverse fossa, on each side of the head, runs upward and backward just in front of the posterior ocelli, but the two do not meet on the middle line.

Color of the body, above, clear brown, the margins, head, and under surface paler.

Length $8^{mm}$; breadth $1^{mm}$.

Station 331, off Cape Cod, in 28 fathoms, 1879.

**Tetrastemma vittatum** Verrill.

American Journal of Science, vol. vii, p. 45, pl. VII, figs. 3, *a*, *b*, 1874; Proc. Amer. Assoc. for Adv. of Science for 1873, p. 389, pl. II, figs. 7, 8, 1874; Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 185, 1879 (not *Œrstedia vittata* Hubr.)

PLATE XXXV, FIGURES 6, 7.

Body rather short and stout, up to 2 or 3 inches in length, soft, changeable, in extension nearly cylindrical but often a little flattened beneath, tapering slightly anteriorly, or sometimes both ways, usually obtuse at the posterior end.

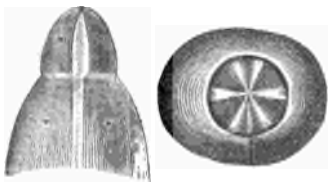


Fig. 6. *Tetrastemma vittatum*; *a*, head, dorsal view; *b*, front view, much enlarged.

Head usually slightly narrower than the body, with a transverse groove or constriction in front of the posterior eyes; front end obtuse, conical, or rounded. Ocelli four, small, rather indistinct in dark colored specimens; the anterior ones are nearer together than the posterior, which are far back behind the transverse groove, which extends across, beneath, and crosses the median line above. Proboscis-pore terminal.

Color of body, above, dark or light olive-green, dull yellowish green, or greenish brown, or even greenish black, often with two yellowish or light green dorsal stripes and sometimes with one median light stripe; beneath paler, mottled laterally. Color of head, in advance of the transverse groove, various shades of olive-green and dark green, white at the tip, and with six longitudinal whitish or pale greenish stripes, which converge to the end; two of these stripes are dorsal, two are ventral, and one is lateral on each side; the green line between the two dorsal stripes extends back on the body. Internal organs show through the integument as irregular, short, transverse blotches or bars of lighter color along the sides beneath.

Length in extension usually about 25 to 30$^{mm}$; diameter 3 to 4$^{mm}$; unusually large examples have been taken as much as 75$^{mm}$ long, 6$^{mm}$ broad. Described from life.

Long Island Sound, Vineyard Sound, Massachusetts Bay, Casco Bay, Bay of Fundy, etc., low-water mark to 25 fathoms, common on muddy bottoms. Noank, Conn., in harbor mud, among eel-grass; Wood's Holl, Mass., harbor mud.

This species is sluggish in its habits and creeps very slowly.

**Tetrastemma vittatum.** Variations.

Several specimens of this species were taken together at station 310, off Cape Cod, in 21 fathoms, 1879.

These show great variation in the color of the body, which, in some was flesh-color, in others light olive-green, dark olive, light greenish brown, dark olive-brown, and dark smoky brown. All these varieties agree, however, in having the head greenish with the six light vittæ distinctly marked. In all the specimens the four ocelli were detected, but they are so indistinct in the dark specimens that they must be sought with care.

The largest specimens were 50 to 75$^{mm}$ long; diameter up to 6$^{mm}$.

**Tetrastemma roseum** Verrill, sp. nov.

Body round and soft, in extension about 1·25 inches long. Head obtusely conical; a transverse shallow groove close to the end of the snout; the part beyond the groove is capable of withdrawing under the portion behind it. Ocelli obscure; two behind the groove and (apparently) two very minute ones in front of it. Color clear bright rosy red.

Length about 30$^{mm}$; diameter 3$^{mm}$.

Station 826, off Block Island, in 22 fathoms, 1880.

This species was met with only once, and then circumstances prevented a careful study of its structure. In the form of the body and characters of the head and cephalic grooves it resembles *T. vittatum* V., and if the front ocelli were correctly noted, would appear to be closely allied to it and might even be thought to be a plain red variety, were not that species very constant in its color markings, the longitudinal vittæ being very characteristic.

**Emplectonema** Stimpson.

Prodromus, in Proc. Philad. Acad. Nat. Sci., vol. ix, p. 164 [20] 1857.

*Amphiporus* Œrsted, Kroyer's Tidds., iv, p. 581, 1844, (? *non* Ehr., *non* McIntosh).

*Nemertes* McIntosh, Nemerteans, p. 176, 1873 (*non* Cuvier, 1817, *non* Œrsted, 1844, *non* Diesing, 1850, *nec* White, 1850).

*Macronemertes* Verrill, Amer. Jour. Sci., vol. vi, p. 439, 1873.

Body much elongated in extension, sometimes almost filiform, very contractile, rounded or a little flattened. Head not very distinctly defined; in some cases with a pair of longitudinal or oblique, shallow, submarginal fossæ on the upper side; in other cases (*E. gracilis*) without evident fossæ.

Ocelli variously arranged, often numerous and in several clusters, both anterior and cerebral.

Proboscis relatively small, especially the anterior portion, which is much shorter than in *Amphiporus*. Mouth usually (always?) separate from the proboscis-pore.

For this genus the earliest available name seems to be that given by Stimpson, who named as type *E. camillea* (Quatr. sp. = *E. Neësii* Œrsted sp.), which is also the type of the genus *Nemertes* of McIntosh. The latter name could not be retained in this sense, even if the genus had not received a prior name, for *Nemertes* had already been used for a genus of insects by White in 1850, in addition to its prior use for several distinct genera of Nemerteans. Had not Stimpson's generic name been available, *Macronemertes* would have been next in order.

## Emplectonema giganteum Verrill.

*Macronemertes gigantea* Verrill, Amer. Journ. Sci., vol. vi, p. 439, pl. 7, figs. 2, *a*, *b*, 1873; Expl. of Casco Bay, in Proc. Amer. Assoc. for 1873, p. 390, pl. 2, figs. 5, 6, 1873.

PLATE XXXV, FIGURE 2. PLATE XXXVIII, FIGURES 12, 12*a*.

Size large. Body much elongated, very contractile; in extension, subterete, a little depressed, thickest anteriorly, gradually tapering posteriorly, becoming very slender and considerably flattened toward the end. Integument very soft, secreting a large quantity of mucus. Head not distinct from the body and of the same diameter, obtusely rounded in front, with a terminal proboscis-pore; upper surface with two shallow, indistinct, sublateral, longitudinal fossæ, often becoming more distinct in alcohol; below with two rather indistinct, obliquely transverse grooves or fossæ.

Ocelli numerous, but not very distinct, because deeply buried in the integument; they are numerous, arranged in four or more clusters; a pair of large oval or subtriangular clusters on the anterolateral border of the head, each of which may be divided into an upper and a lower group, the upper part running backward; a pair of smaller lateral clusters farther back; and a pair of small rather indistinct clusters on the dorsal surface, between the longitudinal fossæ.

Color, when alive, deep salmon or bright orange-red, flesh-color below.

Length of the largest examples, 2 to 3·5 meters, or about 7 to 12 feet, in extension; diameter, anteriorly, 6 to $8^{mm}$, or ·30 of an inch.

When preserved in alcohol this species soon loses all its color, contracts greatly in length, and becomes quite hard; sometimes the body is considerably flattened, but in most cases it retains its subterete form except toward the posterior end. The head often shows the shallow, dorso-lateral, longitudinal fossæ (not slits) and the two large anterior groups of ocelli can usually be seen indistinctly as dark rounded patches beneath the thick outer integument. The small proboscis is often protruded a short distance, its pore being then rather small and slightly below the tip of the snout. The mouth is not visible in the preserved specimens, but my original sketches, made from life, show what I then supposed to be the contracted mouth beneath the head, distinct from the proboscis-pore.

In some horizontal sections of the head the anterior ocelli form an upper pair of transverse groups nearer the front than the sides of the head, while two smaller clusters, lower down in front, seem to be nearly separate from the upper ones. In some specimens these large anterior groups are formed of four or five transverse horizontal rows, of which the upper row runs back to a small lateral cluster of ocelli. The œsophagus has a large plicated anterior portion in the head. The mouth appears to open decidedly behind the proboscis-pore.

In sections of the body the intestine, blood-system, and muscular layers are nearly as in *Amphiporus*, but the muscular layers are unusually thick.

One specimen, taken August 12, 1873, contained large eggs, arranged in about six rows of sacs, above and at the side of the intestine, on each side. The armature of the proboscis has not been observed, but in other respects the proboscis agrees with that of the allied species; the length of the anterior region is about twice the diameter of the body; the posterior portion is long and slender.

Off Cape Elizabeth, 68 fathoms, soft mud, August 12, 1873; Gulf of Maine, 88 fathoms, mud, station 45, 1874; off Martha's Vineyard, 192 fathoms, fine sand and mud, station 869, 1880; off Martha's Vineyard, 229 fathoms, sandy mud, station 925, 1881; off George's Bank, 852 fathoms, gray mud, station 2531; off Block Island, 156 fathoms, fine mud and sand, station 2537; off Block Island, 131 fathoms, fine sand, station 2544, 1885. (U. S. Fish Commission).

### Family, DREPANOPHORIDÆ Verrill, nov.

*Amphiporidæ* (*pars*) authors.

Proboscis-sheath provided with cæcal appendages. Central armature of proboscis a lamelliform plate bearing a number of small stylets on its edge. Lateral stylet-sacs more than two; often numerous, containing small nail-shaped stylets.

### Drepanophorus Hubrecht.

Body and head nearly as in *Amphiporus*; mouth-opening separate from the proboscis-pore. Proboscis large. Musculature of the body-wall as in *Amphiporus*.

### Drepanophorus Lankesteri Hubrecht.

Voyage of the Challenger, vol. xix, pp. 18, 50, pl. i, fig. 22; pl. ix, figs. 1, 2, 10; pl. x, figs. 2, 4; pl. xii, fig. 5; pl. xiv, figs. 9, 10; pl. xv, fig. 13, 1887.

This species was described from alcoholic specimens, destitute of the proboscis. Its external features are, therefore, entirely unknown. Its anatomy was, however, carefully worked out by means of sections.

It is peculiar in having numerous well marked transverse nervous commissures connecting the lateral nerve-trunks anteriorly.

The cæcal appendages of the proboscis-sheath are also unusually well developed, with thicker walls than in most species, and they sometimes anastomose distally.

Ocelli are present, but their arrangement was not stated. Genital sacs are numerous, apparently in four rows, subventral.

For other details reference should be made to the original description and figures.

Off Nova Scotia, near Le Have Bank, 45 fathoms.

I have observed a single, small, and probably immature, specimen of DREPANOPHORIDÆ on the New England coast, but do not deem it wise to name it. This was translucent yellowish white in color.

## Order II, ANOPLA.

*Anopla* Max Schultze, 1852; McIntosh.

Proboscis unarmed, long, slender, tubular, and not divided into three distinct regions; its walls may contain three to five layers; inner surface, when retracted, papillose.

Head with or without lateral slits or ciliated pouches.

Ocelli variously arranged; often wanting.

Mouth ventral, situated behind the ganglions.

The muscular walls of the body often consist of three layers: outer longitudinal, middle circular, and inner longitudinal (Pl. xxxix, figs. 17 to 21). In some cases the outer longitudinal layer is lacking (figs. 15 and 16).

Two or three main vascular trunks; the vessels generally not so well defined as in the Enopla, and often having in part the character of wide lacunæ, especially anteriorly.

Œsophagus entirely behind the brain, usually large, long, plicated, and surrounded by a vascular network, or by lacunæ. (Pl. xxxix, fig. 22, *r*, *r*).

Lateral nerve-trunks arise from the outer sides of the lower ganglions, and are situated between the muscular layers of the body-walls, but they vary in position in the different families. Usually there is a nervous plexus outside of the circular muscular layer. (Pl. xxxix, figs. 17, 20, *n*).

The species are almost all marine; a few inhabit brackish water.

## Suborder I. RHAGADOCEPHALA Diesing, 1850 (emended).

*Schizonemertini* Hubrecht; Carus, Faunæ Med., p. 160.
*Schizonemertea* Hubrecht, Voy. Challenger, xix, p. 37.

Head with a deep, longitudinal, ciliated slit, or fossa (probably olfactory in function), on each side, terminating posteriorly in a deep pit or duct running inward to the posterior ganglions. (Pl. xxxix, fig. 22, *f*, *d*, *d'*).

Mouth large, behind or opposite the posterior ends of the lateral slits and cephalic ganglions. (Wood-cut 8).

Lateral nerve-trunks situated between the outer longitudinal and the circular muscular layers of the body-wall. (Pl. xxxix, figs. 19 to 21). A median dorsal nerve is also usually distinctly developed.

Three large, longitudinal, vascular trunks, which are usually connected by numerous transverse vessels around the intestine, especially posteriorly.

Œsophagus large, prolonged backward, plicated, and provided with a vascular system, probably having a respiratory function. (Pl. xxxix, figs. 20, 22).

Many of the species of this group develop directly from the eggs, without a marked metamorphosis, but certain species of *Micrura* (perhaps all) have a peculiar, free-swimming larval form known as *Pilidium* (Pl. xxxix, figs. 1 to 6, and wood-cut 7). The embryology of the closely related genus, *Cerebratulus*, is apparently unknown.

The species are almost exclusively marine and are found in deep water as well as between tides. Many are fossorial in their habits,

Fig. 7. Pilidium of *Micrura*, much enlarged: *c*, apical cilium; *h*, cephalic lobe; *m*, mouth; *i*, intestine; *v*, bands of cilia; *o*, young nemertean developing in the interior, showing its head with two ocelli.

living in sand or mud, or beneath stones. Some of the large flat species of *Cerebratulus* leave their burrows and swim with an undulatory, eel-like movement at the surface of the sea at night.

## Family, Lineidæ McIntosh.

Body simple, generally much elongated in extension, very contractile, usually thickest in the region of the œsophagus, and becoming

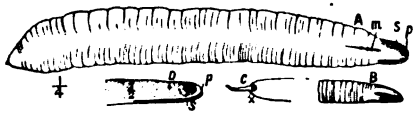


Fig. 8. *Lineidæ*. A, *Cerebratulus luridus*, ventral side; *m*, mouth; *s*, one of the olfactory slits or cephalopori; *p*, proboscis-pore. B, head of the same, side view. C, tail; *x*, anus. D, head of *Lineus* viridis, young, enlarged; *s*, one of the cephalopori; *p*, proboscis-pore.

more or less flattened farther back, where the saccular appendages of the intestine and the reproductive glands occupy the sides. Head simple, with elongated lateral olfactory slits, or cephalopori.

### Lineus Sowerby, 1806.

*Lineus* Sowerby, British Miscel., p. 15, pl. 8, 1806.
*Borlasia* Oken, Lehrbuch, p. 365, 1815; Blainville, Dict. Sci. Nat., 57, p. 575, 1828; Johnston, Catal., p. 21, 1865.
*Nemertes* Cuvier, Regne Anim., vol. iv, p. 37, 1815; Dies. (*pars*), op. cit., p. 264.
*Lineus* Œrsted, Naturh. Tidsskr., iv, p. 576, 1844.
*Meckelia* (*pars*) Diesing, Syst. Helm., vol. i, p. 265, 1850.
*Notospermus* Diesing, op. cit., vol. i, p. 260.
*Lineus* Stimpson, Prodromus, p. 160, 1851.
*Cerebratulus* (*pars*) Stimpson, Prodromus, p. 160, 1857.
*Poseidon* Girard, Proc. Boston Soc. Nat. Hist., iv, p. 185, 1852.
*Nemertes* Verrill, Invert. Vineyard Sound, etc., 1873.

Body very contractile, in extension elongated, slender, tapering, and often attenuated toward the posterior end, rounded or slightly depressed anteriorly, generally somewhat broader and more depressed in the middle region, but without the conspicuous flattening back of the œsophageal region seen in *Cerebratulus*. No anal papilla. Head elongated, not very distinctly defined; often a little wider than the neck, but not constantly so. Lateral slits elongated and deep, running close to the terminal proboscis-pore, but usually not joining it. Mouth, in ordinary states, rounded or elliptical and not very large, but capable of great extension when feeding. Ocelli small, usually arranged in a simple row along the lateral margins of the head, sometimes absent.

The several European species of this genus have been referred by authors to a great number of different genera, of which I have indicated only a part. The first three names cited in the synonymy were all given to the same species (*L. marinus* = *L. longissimus*) of Europe and are, therefore, exact equivalents. The two later names should, therefore, have been dropped entirely from the nomenclature of the group. Unfortunately several different writers have tried to restrict both *Borlasia* and *Nemertes* to groups entirely different from that to which they were originally given, and have thus introduced great confusion. Each attempt of this kind has, hitherto, been a failure for in most instances the new groups thus named have been found to have had other and earlier names. One of the latest reapplications of *Nemertes* to a newly constituted group was by McIntosh (Nemerteans, p. 176). He applied it to a genus of *Enopla*, in a wholly new sense. *Nemertes* of McIntosh, 1879, is, however, antedated by *Nemertes* of White, 1860, applied to a Crustacean, and therefore it could not be retained, even if the nemertean genus, so named, had not already received other names.

The use of *Borlasia* by McIntosh, in a wholly new sense, seems also to be untenable.

### Lineus viridis (Fabr.) Johnston.

*Planaria viridis* O. Fabricius in O. F. Müller, Zool. Dan. Prod., 2684, 1776; O. Fabricius, Fauna Grœnlandica, p. 324, 1780; Müller, Zoölogia Danica, ii, p. 35, pl. 68, figs. 1 to 4, (from Greenland specimens sent by Fabricius to Müller).

*Planaria Gesserensis* Müller, Zoöl. Danica, ii, p. 32, pl. 64, figs. 5 to 8, 1788.

*Nemertes olivacea* Johnston, Mag. of Zoöl. and Botany, vol. i, p. 536, pl. 18, fig. 1, 1837; Diesing, Syst. Helm., i, p. 273, 1850.

*Nemertes obscura* Desor, Boston Journal of Natural History, vol. vi, pp. 1 to 12, plates 1 and 2, 1848 (embryology).

*Polia obscura* Girard in Stimpson's Marine Invertebrates of Grand Manan, p. 82, 1853 (no description).

*Nemertes viridis* Diesing, Sitzungsberichte der kais. Akad. der Wissenschaften, vol. xlv, p. 305, 1862.

*Borlasia olivacea* Johnston, Catalogue British Non-parasitical Worms, p. 21, pl. 2[b], fig. 1, 1865; McIntosh, Trans. Roy. Soc. Edinb, vol. xxv, pt. ii, p. 371, 1869.

*Lineus viridis* Johnston, Catal., pp. 27, 296, 1865.

*Nemertes viridis* Verrill, Marine Invert of Vineyard Sound, etc., p. 334 [628], 1873.

*Lineus Gesserensis* McIntosh, Hist. British Annelida, Part I, Nemerteans, (Ray Society) p. 185, pl. iv, fig. 2; pl. v, fig. 1, (red var.); pl. xviii to xxii, (anatomy); pl. xxiii, (green var., embryology), 1873.

*Lineus viridis* Verrill, Check List Marine Invert., p. 12, 1879; Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 185, 1879.

PLATE XXXVII, FIGURES 5 to 5*b*; PLATE XXXVIII, FIGURES 6 to 6*d*; PLATE XXXIX, FIGURES 18, 22.

Body soft, very contractile and changeable; in full extension elongated and moderately slender, in large examples six to eight inches long and about one-fifth of an inch in diameter; usually thickest in advance of the middle, tapering gradually to the rather slender caudal portion, and decreasing less toward the head; not unfrequently the body is dilated in two or more places at the same time, the swollen parts moving continually; in extension the body is usually somewhat flattened, but the dorsal surface is decidedly convex and the sides well-rounded; it is often crossed by faint, light-colored, irregularly spaced wrinkles. In contraction the body becomes short and thick, oblong, swollen and almost saccular at times.

Head, in extension, rather large, depressed, usually wider than the neck, short, ovate-spatulate, or elongated, according to the degree of extension; the snout is blunt, often emarginate, and bears three small ciliated papillæ; proboscis-pore terminal, rounded, or in the shape of a short vertical slit; lateral fossæ long and very deep, with wide, thin, pale margins, above and below, the anterior ends of the slits reaching close to the proboscis-pore.

The ocelli are arranged in a simple row on each side of the head, close to the edge of the dorsal pigmented region; they vary in number and size according to the age, the large specimens often having six or eight on each side, while the small ones have but three or four, and the very young ones have only a single pair; usually the anterior ocelli are slightly larger than the others.

The mouth is situated opposite to, or a little behind, the posterior ends of the lateral fossæ; it is ordinarily small and elliptical, with a distinct, lighter colored border, but it is capable of great dilation

when the creature is engaged in swallowing some annelid nearly or quite as large as itself.

The color, in life, is variable; the most common variety is dull green, or olive green, varying to dark smoky green or greenish black, darkest anteriorly, and with the under surface and caudal portion somewhat paler; region of the cephalic ganglions and lateral pits usually reddish; front and margins of head pale or whitish; on many specimens faint pale transverse lines or rings can be seen, if carefully examined; at times a row of small whitish spots, corresponding to the genital pores, can be seen on each side. Other specimens occur, often living with green ones, in which the general color of the body is brown, greenish brown, reddish brown, or clear red with the margins of the head and lower surfaces flesh-color or reddish.

Some of these forms differ so much in appearance from the common dark green variety that it would be convenient to distinguish them by variety names, using, in this sense, some of the numerous names applied by the early writers when they were supposed to be distinct species, viz:

Var. *olivaceus* (Johnston). The typical green and olive-colored variety.

Var. *fuscus*. The brown and reddish brown variety.

Var. *rufus* (Rathke). The distinctly red variety.

Var. *obscurus* (Desor). The smoky green and blackish variety.

Specimens intermediate in color between all these are, however, of frequent occurrence.

The length, in extension, is sometimes $150^{mm}$ to $200^{mm}$; the diameter $2^{mm}$ to $4^{mm}$; in contraction the body becomes much shorter and stouter, large specimens often being only $30^{mm}$ or $40^{mm}$ long and $4^{mm}$ to $6^{mm}$ broad.

In alcoholic specimens the body is usually thickened and rounded anteriorly, more slender and somewhat flattened farther back, often acute at the posterior end; head obtusely rounded or sub-truncate, with a small terminal proboscis-pore and two lateral slits, which are short and extend forward very near to the proboscis-pore. Mouth small and round, situated slightly behind the posterior ends of the lateral slits; ocelli not apparent. When placed in alcohol the body usually contracts so violently that it breaks up into segments, especially posteriorly, and the proboscis is often completely ejected.

The extruded proboscis is long, slender toward the base, clavate toward the end, the terminal portion transversely wrinkled.

This common littoral and shallow water species is found on the American coast from Long Island Sound to Labrador, Cumberland Gulf, and Greenland. It is also common on the coasts of Great Britain as far south as the Channel Islands (McIntosh), and on all the northern coasts of Europe.

South of Cape Cod, I have collected it near New Haven, Conn., and at the Thimble Islands; Noank, Conn.; Newport, R. I.; Wood's Holl, Mass.; and at various other localities. North of Cape Cod it is more abundant and larger. Among the localities where I have taken it are Provincetown and Barnstable, Mass.; Salem and Gloucester, Mass.; Casco Bay; Mount Desert Island; Eastport, Me.; Grand Menan Island; Halifax, N. S.; Gulf of St. Lawrence, etc.

It is particularly abundant and large at Eastport, Me., and at all localities about the Bay of Fundy, where the shore is composed of rocks.

This species is active and restless in confinement. It creeps rather rapidly and is prone to climb out of the water and perish by drying up. It is a voracious feeder and lives largely upon annelids. I have observed it in the act of swallowing a full grown scaly annelid, (*Lepidonotus squamatus*), which was considerably greater in diameter than the thickest part of its own body. A specimen of this kind, with the *Lepidonotus* half swallowed, is preserved in the museum of Yale University.

The eggs are deposited in great abundance on our shores under stones near low-water mark, in midsummer. They are contained in more or less cylindrical masses of a translucent, dull greenish, jelly-like substance, made up of numerous capsules, (Plate XXXVIII, fig. 6*c*). These cylinders are usually from $3^{mm}$ to $5^{mm}$ in diameter, and $40^{mm}$ to $50^{mm}$ in length, and are usually coiled in a spiral or ring-like form. The eggs are in several rows. In those clusters taken at one date, in July, at Eastport, Me., I have found eggs in all stages of development, while in some of them the recently hatched young were still present. (Pl. XXXVIII, fig. 6*d*).

I have adopted the name, *viridis*, given to this species by Otho Fabricius, who communicated the first published descriptions and figures to Müller, as stated both by him and by Müller. That this is the species observed on the shores of Greenland and described by Fabricius there can be no reasonable doubt. His brief description is quite as correct and characteristic as the descriptions of such animals were wont to be at that time, and his figures, published by Müller in the Zoölogia Danica, represent the worm fairly well when

partially contracted; nor could they be referred to any other Greenland species.

The lateral slits of the head of this species are spoken of on p. 325 of the Fauna Grœnlandica, and are also distinctly shown in the figures. Fabricius speaks of his *viridis* as common on the shores of Greenland among the roots of algæ. I have personally examined good specimens of this species recently taken on the coast of Greenland in the same situations. There can be no doubt of their identity with the true *viridis*. Therefore there is not the slightest reason why his characteristic name should not be used, in preference to *Gesserensis*,* of much later date.

Although the latter name has been adopted by many recent writers on European nemerteans, the ordinary rules of priority, as well as justice to the very meritorious author of the Fauna Grœnlandica, should compel a change in this respect.

Fabricius and Müller, in the same works, described another Greenland form under the name of *rubra*. I am of the opinion that this was simply the red variety of *L. viridis*, for the two varieties occur together everywhere on the northern coast of America. Levinsen, however, has referred the *rubra* to *L. sanguineus*, and has given the latter as a Greenland species. If both species actually inhabit Greenland his view may be correct, for there is nothing in the original description to indicate that it belongs to one rather than to the other of these two species, if it really belonged to either.†

Indeed these two reputed species are so much alike that I am myself in doubt whether they are really distinct. There is no special diagnostic character given by McIntosh unless it be the somewhat narrower head in *L. sanguineus*. The shape of the head in this genus is so changeable and variable that, in practice, little reliance can be placed upon this as a diagnostic character. The ocelli are supposed to differ slightly in size and number, but they also vary in both forms. Hence it seems to me not improbable that a more extended study of the variations will compel us to consider *L. sanguineus* only a lighter red variety of *L. viridis*. In this article I have, however, followed most European writers in keeping them separate, although I confess that with scores of living specimens of both

* Levinsen, in his recent paper on the Turbellaria of Greenland, also records the typical form, under the name of *L. Gesserensis*, as a native of that coast.

† Fabricius mentions neither ocelli nor cephalic slits in this species. Therefore it may not have been a *Lineus*. The necessary doubt concerning its true relations should prevent the application of the name to any species.

forms before me, I have always found it difficult to draw any clear line of separation between them.

## Lineus sanguineus (Jens Rathke).

*Planaria sanguinea* Jens Rathke, Skrivter af Naturhist. Selsk., vol. v, i, p. 83, 1799.
*Planaria octoculata* Johnston, Zoöl. Jour., vol. iv, p. 56, 1829.
*Nemertes (Borlasia) octoculata* Johnston, Mag. Zoöl. and Bot., vol. i, p. 537, pl. 18, fig. 2, 1837; Œrsted, Kroyer's Naturhist. Tidss., iv, p. 579, in note, 1837.
*Nemertes sanguinea* Œrsted, Entw. Plattw., p. 92, 1844.
*Borlasia octoculata* Johnston, Catalogue Brit. Mus., pp. 21, 287, 290, pl. II*b*, fig. 2, 2*, 1855.
*Lineus sanguineus* McIntosh, British Annelids, part I, Nemerteans, p. 188, pl. v, fig. 2, 1873.

PLATE XXXVIII, FIGURES 10, 10*a*.

Body strongly convex or well rounded above, flatish beneath, rather long, in extension often 8 to 10 inches long and .25 inch broad, but it is capable of contracting to less than one-fourth this length, and then becomes about .35 of an inch in diameter. Head elongated, usually not so wide as the body, often acute in front when extended, but it changes much in form and may become much shorter and obtuse in contraction; lateral cephalic slits, moderately long and deep, bordered by narrow pale lips, above and below. Ocelli small, but very distinct, blackish, usually 4 to 8 in a row on each side, arranged at the upper margin of the white lateral borders of the head. Mouth rather large, usually round or oval, with corrugated white lips, but capable of great extension when swallowing large prey.

Color of body, above, dark red, bright red, or clear reddish brown, usually darker medially; beneath, pale salmon, flesh-color, or light yellowish red; snout and margins of head whitish; the red color of the middle of the head slightly emarginate or notched at its anterior end.

Eastport, Me., at Dog Island, low-water, under stones, 1868, (No. 2). Also taken at various other localities at Eastport and Grand Menan, between tides, in 1870 and 1872, common.

Under *L. viridis*, on a previous page, I have spoken of the close relationship of the red variety of that species to *L. sanguineus*, and have given reasons for doubting the status of this as a distinct species—at least as they occur on our northern coasts. It may be possible that we do not have the real European *L. sanguineus*, but we have an abundance of specimens that agree in all respects, so far

as I can see, with the descriptions and figures given by McIntosh and others.

The character upon which McIntosh puts the most stress is the greater narrowness of the head, said to be chiefly due to the narrower lips of the cephalic slits in the present species, as compared with *L. viridis* (*Gesserensis*). But as the length and breadth of the head and of the margins of the slits are constantly changing during the motions of the living worms, it is not easy to make sure of such differences. The lighter and brighter red color of the body, and the greater distinctness of the ocelli in *L. sanguineus* are also supposed to be characteristic.

It is found chiefly under stones from half-tide to low-water mark, and at moderate depths (1 to 25 fathoms) on stony and muddy bottoms. Many specimens are often found living gregariously, curled up together, under one stone.

### Lineus socialis (Leidy) Verrill.

*Nemertes socialis* Leidy, Marine Invert. Fauna of Point Judith, R. I., and New Jersey, p. 11 [143], 1855; Verrill, Invert., Vineyard Sd., etc., p. 334 [628].

*Lineus communis* Van Beneden (?); Verrill, Notice of Recent Addit. to Mar. Invert. Part I. in Proc. National Mus., ii, p. 185, 1879.

PLATE XXXVII, FIGURES 8, 8*a*; PLATE XXXVIII, FIGURES 7, 7*a*.

Body very long and slender, subterete, attenuated posteriorly, in full extension almost linear, up to 8 to 10 inches long, with the diameter about .04 inch. Head very long, flattened, obtuse; lateral cephalic slits very much elongated. Mouth placed far back from the front of the head. Ocelli very small, often obscured by the dark color of the head, in large specimens four to six or more in a row on each side of the head, the front pair larger than the others and usually separated by a slightly greater interval; very young ones have only a single pair. Color, above, usually dark olive-green, greenish brown, greenish black, or smoky brown, and more rarely reddish brown, the anterior parts often darkest; indistinct, rather distant, pale transverse lines are often present, and occasionally there is a darker median dorsal stripe; front margin of the head paler and slightly translucent; lower surface of the body usually similar in color to the back but of a paler shade, most frequently dull green or greenish ash.

Length of large specimens, in extension, $250^{mm}$, diameter $1^{mm}$ to $5^{mm}$.

This is a strictly littoral species. It is common from New Jersey to the Bay of Fundy. It occurs abundantly and usually gregarious-

ly under stones, among living mussels, between the roots of grasses and algæ, etc., from near low-water mark nearly up to high-water mark of medium tides. I have collected it at Great Egg Harbor, N. J.; New Haven, Conn.; Noank, Conn.; Newport, R. I.; Wood's Holl, Mass.; in the harbors of Provincetown, Barnstable, Salem, and Gloucester, Mass.; Portland and Eastport, Me., etc.

This species is very gregarious, a large number usually living coiled up together in a tangled mass, from which, however, the individual worms can easily disengage themselves when disturbed. It occurs nearly up to high-water mark where other nemerteans are not found.

Superficially this species resembles, in color and general appearance, the young of *L. viridis* (dark green variety), but it is relatively much longer and more slender, and has a much longer and narrower head, with decidedly longer lateral slits, and the mouth is placed much farther back.

### Lineus arenicola Verrill.

*Tetrastemma (?) arenicola* Verrill, Invertebrate Animals of Vineyard Sound, etc., p. 335, pl. xix, fig. 98, 1873.

PLATE XXXVIII, FIGURES 5, 5*a*.

Body subterete, long, slender, slightly depressed, of nearly uniform width ; the head is very versatile, usually sub-conical or lanceolate, flattened, occasionally becoming partially distinct from the body by a slight constriction at the neck. Ocelli four, those in the anterior pair nearer together. The lateral fossæ are long, and deep slits on the sides of the head. Mouth small, often sub-triangular, situated just back of the posterior ends of the lateral fossæ. Body deep flesh-color or pale purplish.

Length about $100^{mm}$, in extension.

Savin Rock, near New Haven, Conn., in sand at low-water mark.

This species has not been taken except in the original locality. It appears to be very rare in our waters. Possibly it is a southern species that does not ordinarily live so far north.

### Lineus pallidus Verrill.

*Lineus pallidus* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 186, 1879.

PLATE XXXVII, FIGURES 9, 9*a*.

Body long and very slender in extension, subterete, attenuated posteriorly. Head elongated, usually obtuse and wider than the

body, but very changeable. Ocelli absent. Lateral (cephalic) fossæ long and deep. Mouth situated far back from the anterior end. Color usually whitish or pale ocher-yellow, usually becoming reddish toward the head, and with a rather indistinct pale dorsal line; anteriorly there are usually two pale dorsal spots in front of which the head is yellowish.

Length, in extension, 100$^{mm}$; breadth 0·5 to 0·75$^{mm}$.

Off Cape Ann, Mass., 45 fathoms, mud, 1878.

## Lineus dubius Verrill.

*Lineus dubius* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p, 186, 1879.

PLATE XXXVII, FIGURES 4, 4*a*.

Body very slender in extension, and attenuated posteriorly. Head elongated, narrow, usually pointed; lateral slits of moderate length; ocelli *white*, inconspicuous, forming a lateral row of about twelve, extending back on each side of the head beyond the posterior ends of the lateral fossæ, usually the four anterior ones on each side are separated by a little space from those that follow, but all are nearly in a simple row. Color, above, light green to dark olive-green.

Length of the largest specimens observed, 50 to 75$^{mm}$.

Gloucester, Mass., under stones, between tides, 1878.

## Lineus bicolor Verrill, sp. nov.

PLATE XXXVII, FIGURES 8, 8*a*, 8*b*.

Body rather small, in extension elongated, thickest and somewhat depressed in the middle, tapering to both ends, and decidedly attenuated posteriorly; sides rounded. Head elongated, flattened, rather wider than the neck, in usual extension. Lateral olfactory slits long and deep, with thin margins. Mouth usually elliptical, situated behind the ends of the olfactory slits. The ocelli are arranged in a simple row of about 4 to 7 on each side of the head, the front pair largest. Color, above, along each side of back a broad stripe of olive-green, yellowish green, or brownish green, separated by a median dorsal, well defined, broad stripe of pale yellow or yellowish white, usually becoming clear white on the head, where it expands and blends with a white frontal area in advance of the eyes; the margins of head are also white. Lower surface pale greenish or yellowish white.

Length in extension, 35 to 45$^{mm}$; diameter, 1 to 1.5$^{mm}$. Described from life.

Long Island Sound to Vineyard Sound, in 2 to 24 fathoms; Bartlett's Reef, 22 fathoms, 1874. Usually taken on shelly or stony bottoms among algæ, ascidians, and hydroids; common, especially in Vineyard Sound. The specimen figured was taken at Wood's Holl, July 14, 1875.

DOUBTFUL SPECIES.

**Lineus truncatus** (Hubr.) Verrill.

*Cerebratulus truncatus* Hubrecht, Voyage of the Challenger, vol. xix, pp. 37, 50, pl. 1, figs. 11, 12, 1887.

This species was described from imperfect alcoholic specimens, so that its external form and color in life are entirely unknown.

In the contracted specimens the head is short, flattened, truncated in front; the cephalic slits are short and run forward close to, but do not join, the proboscis-pore; the mouth is small, rounded, and only a short distance back from the front.

Off Nova Scotia in 75 and 85 fathoms; also off Bermuda. (Challenger Exp.)

The small mouth, rounded body, and general appearances of the specimens, as figured, indicate that it belongs to *Lineus* or *Micrura*, and not to *Cerebratulus*, as here defined. There is nothing in the description to distinguish it from *Lineus viridis*, which often contracts into the same form.

**Micrura** Ehrenberg, 1830.

*Micrura* (*pars*) McIntosh, Nemerteans, p. 196.
*Cerebratulus* (*pars*) Hubrecht.

Body, head, and proboscis nearly as in *Lineus*; body elongated, terete or somewhat flattened; the posterior regions usually not much flattened, nor very different in form from the region of the proboscis. Cephalopori or olfactory slits well defined. Ocelli sometimes present, but often wanting. Posterior end of the body provided with a median slender cirrus, above the anus.

This genus, as here defined, differs from *Lineus* in little else than the presence of a well marked contractile anal cirrus, which may often be distinguished even in alcoholic specimens. From *Cerebratulus*, which also has the anal cirrus, it differs in the form and muscular structure of the body posteriorly, which is not very flat and thin, nor adapted for swimming, as in the latter.

Some of the flat species included in this genus by McIntosh, I should, therefore, transfer to *Cerebratulus*, especially his *M. fusca* (*non* Fabr. sp.)

Hubrecht has united *Micrura* and *Cerebratulus* without regard to the form of the body and the muscular structure of the body-walls, which seem to me important characters, involving wide differences in habits.

The species of *Micrura* are fossorial in their habits and do not swim at the surface, so far as I have observed, and, indeed, the form and structure of the body are not adapted for swimming.

Some of the species of *Micrura*, if not all, have a Pilidium-stage in development. The embryology of many of the species has, however, not been traced. Nor have any of the several species of Pilidium-larvæ found on our coast been reared till the adult characters could be determined. On Plate XXXIX, I have figured two distinct kinds of these larvæ that are common at Wood's Holl, Mass., in summer. One or both probably belong to some of our species of *Micrura*, but as the larval form of *Cerebratulus* is unknown, one of them may belong to that common genus. The young nemertean seen in the interior of one species (fig. 5, *w*), has already two distinct ocelli, which would indicate that it belongs to a species like *M. affinis*, which has ocelli when adult.

## Micrura affinis Verrill.

*Poseidon affinis* Girard in Stimp., Marine Invert. of Grand Manan, p. 28, 1853.

*Nemertes affinis* Verrill, Amer. Journ. Sci., vol. vii, pp. 39, 412, 1874; Proc. Am. Assoc., for 1873, pp. 351, 363.

*Micrura affinis* Verrill, Proc. U. S. Nat. Mus., vol. ii, p. 186, 1879; Check List, Invert., p. 12, 1879.

PLATE XXXVI, FIGURE 1; PLATE XXXVII, FIGURES 6, 6*a*.

Body elongated in extension, somewhat depressed, but with rounded sides, of nearly uniform breadth through most of the length, but somewhat tapered posteriorly, and terminated by a slender, pointed, pale anal papilla or cirrus, about one-half as long as the diameter of the body. Head scarcely wider than the neck, elongated, flattened, usually obtusely rounded anteriorly, but changeable. Lateral olfactory slits long and deep, with thin white margins in front, uniting with the proboscis-pore. Mouth of moderate size situated opposite the ends of the slits. Ocelli rather large, black, conspicuous, variable in number, forming a single row, usually of four to six on each side at the edges of the white marginal areas; the front ocelli are

usually distinctly larger than the others. Color, above, usually clear bright red, varying to dark red and reddish brown, rarely to greenish brown; often crossed by indistinct, transverse, pale lines, as in *Lineus viridis*; front and margins of head white; under surface light flesh-color or pinkish, often showing by translucency the intestinal cæca or pouches along the sides in the form of transverse gray blotches.

Length up to 125 to 150$^{mm}$; diameter, 2 to 4$^{mm}$. Described from life.

Very common from off Cape Cod and Massachusetts Bay to Nova Scotia, in 8 to 150 fathoms or more, on shelly and stony bottoms. It is particularly common in the Bay of Fundy, the harbor of Eastport, Me., and the other cold waters of that region, where it is also often found at low-water mark under stones. It has also been frequently dredged in 12 to 50 fathoms south of Cape Cod, and off Nantucket and Martha's Vineyard, in the cold area.

This species, in some of its red and brown varieties, closely resembles the red and brown varieties of *Lineus viridis*, from which it cannot be distinguished when living without a careful examination. The presence of the caudal cirrus is easily diagnostic, when the specimen is perfect, but when mutilated, as often happens, the difficulty is much increased. The ocelli in this species are usually larger and more distinct than in *Lineus viridis*.

**Micrura dorsalis** Verrill, sp. nov.

PLATE XXXVIII, FIGURES 4, 4*a*.

Body depressed, rather large and thick, length up to 6 inches in ordinary extension; in contraction it becomes short and thick, and may even contract itself into a ball; the margins are well rounded and the body tapers toward both ends. The anterior region of the body for about a sixth to a tenth of the whole length, often becomes in partial contraction rounded and narrower than the rest of the body. Head obtusely pointed or bluntly rounded in front, not distinct from the body and of the same breadth. Cephalopori moderately long, somewhat oblique longitudinal slits on the sides of the head, extending nearly to the proboscis-pore. Ocelli, none. Mouth small, rounded, nearly opposite the hind end of the cephalopori.

Color pale ocher-yellow with an orange tinge anteriorly, with a darker medial stripe above and below, and having pale mottlings indistinctly showing through on each side due to the internal organs.

Length 160$^{mm}$; diameter 5$^{mm}$. Described from life.

The type-specimen, described above, as now preserved in alcohol, has a stout body, thickest anteriorly, tapering to the hind end, which terminates in a small, whitish caudal papilla. The sides are everywhere rounded. Head short, thick, subconical, blunt, not distinct from the body; proboscis-pore terminal, in the form of a short vertical slit; lateral cephalic slits moderately long, joining the proboscis-pore in front, so as to divide the tip of the snout into four parts. Mouth small, rounded, opposite the posterior ends of the cephalic slits.

Length in alcohol, $64^{mm}$; diameter $3^{mm}$; length of cephalic slits $3^{mm}$.

Eastport, Me., at Clark's Ledge, extreme low-water mark, under stones, 1870.

**Micrura rubra** Verrill, sp. nov.

PLATE XXXVIII, FIGURES 3, 3*a*, 9, 9*a*.

Body moderately large, subterete and elongated in extension, up to 3 inches long, rather more slender posteriorly. Head obtuse or rounded in front; proboscis-pore a vertical terminal slit; cephalic slits or cephalopori long and deep, in front joining the proboscis-pore so as to divide the tip of the snout into four small lobes; the slits extend back as far as, or beyond, the mouth, which is ordinarily a small elliptical opening. No ocelli.

Color, above, light orange red to bright red, indistinctly mottled along the sides with brownish red, due to internal organs.

Length 62 to $75^{mm}$ in extension; diameter $2{\cdot}5^{mm}$. Described from life. (No. 722).

In alcohol the specimens above described are much contracted, thick and short, stoutest anteriorly, tapered, but scarcely flattened posteriorly. Ovaries filled with eggs commence some distance back of the head. Cephalic slits moderately long and deep, joining the proboscis-pore in front. Prosboscis, as ejected, coiled in a spiral, moderately long and rather thick, tapering to both ends.

Off Casco Bay, July 16, 1873.

A curious specimen (Plate XXXVIII, fig. 3, 3*a*), probably of this species, was taken in the Bay of Fundy. It had, apparently, been broken and was in the act of reproducing the hinder part of the body.

Body round, cylindrical in extension, very changeable in shape: posterior end abruptly narrowed into a small, round caudal portion terminating in a small papilla. Head obtusely rounded or obliquely

conical with an oblique lateral cephalic slit on each side; mouth small, opposite the posterior ends of the slits. No ocelli.

Color nearly uniform deep flesh-color. The salmon-colored ovaries show through slightly, especially posteriorly, as transverse spots.

Length about $25^{mm}$; diameter $2{\cdot}5^{mm}$.

Bay of Fundy, off Head Harbor, Campo Bello Island, 40 fathoms, mud, Aug. 27, 1870. (Catal. No. 117).

### Micrura albida Verrill.

*Micrura albida* Verrill, Notice of Recent Addit. to Mar. Invert., Part I. in Proc. National Mus., ii, p. 186, 1879.

Body slender, thickest and nearly round anteriorly, gradually tapered and somewhat flattened posteriorly, with a small, slender caudal papilla. Head flattened, narrow, obtuse, narrower than the body. No ocelli. Lateral fossæ rather short, extending beyond the mouth, not conspicuous. Color whitish, or pale yellowish, often becoming light red toward the head; posteriorly often with grayish or clay-colored internal mottlings along the sides, due to the reproductive organs. Very sluggish in its motions.

Two specimens from 140 fathoms, off Cape Ann, apparently of the same species, were milk white above, with fine specks of opaque white, the ganglions showing as red spots; they had a narrow but distinct ring of blue around the body, behind the head.

Length, 50 to $125^{mm}$; diameter 2·5 to $3^{mm}$.

Common in the Gulf of Maine and Massachusetts Bay, on muddy bottoms, in from 30 to 140 fathoms.

It is sluggish in its movements and constructs translucent tubes of tough mucus.

### Micrura inornata Verrill.

*Micrura inornata* Verrill, Notice of Recent Addit. to Mar. Invert., Part I, in Proc. National Mus., ii, p. 186, 1879.

PLATE XXXVII, FIGURE 7.

Body subterete, moderately elongated, thickest anteriorly or in the middle, gradually tapered to the somewhat flattened tail; caudal filament white, very slender and acute, sometimes as long as the diameter of the body, but usually less. Head obtuse, often as wide as the body or wider, somewhat flattened. Lateral fossæ long, deep, curved, extending to opposite the mouth, the latter not being very far back. No ocelli. Color above, bright cherry-red, varying to dark red, the middle of the head brightest; tail pale.

Length of largest specimens observed, about $75^{mm}$; breadth, 1·5 to $2^{mm}$ in extension. Described from life.

Massachusetts Bay and Gulf of Maine, 45 to 110 fathoms, mud.

Resembles the young of *Cerebratulus luridus V.*, which occurs with it.

## Cerebratulus Remer, 1804.

*Meckelia* Leuckart, Breves Anim., p. 17, 1828 (t. Rathke); Diesing, Syst. Hel. vol. i, p. 266, 1850.

*Serpentaria* Goodsir, Ann. Nat. Hist., vol. xvi, p. 377, 1835.

*Cerebratulus* (*pars*) and *Meckelia* Stimpson, Prodromus, p. 160, 1857.

*Cerebratulus* McIntosh, British Annelids, Part I, Nemerteans, p. 194, 1873.

*Cerebratulus* (*pars*) Hubrecht, Voy. Challenger, vol. xix, p. 37, 1887; Carus, Fauna Medit., p. 160.

Body large, elongated, much flattened along the middle and posterior portions and adapted for swimming by having the margins produced and thin, mainly owing to the unusual development of the longitudinal muscular layers, which are greatly thickened, especially the outer layer, which, as seen in transverse sections, forms a more or less triangular band, much thicker than elsewhere, (Plate XXXIX, fig. 19, *l*). Transverse muscular bundles running from the upper to the lower sides of the inner surface of the body-wall (fig. 19, *t'*) are also unusually well developed so as to aid in giving an undulatory motion to the margin while swimming.

Anterior or œsophageal region large, with rounded margins (fig. 20). Cæcal appendages of intestine numerous and crowded, elongated, more or less forked and lobed at the outer ends, the divisions occurring partly horizontally, and showing well in sagittal sections.

Head versatile in form, usually without ocelli. Cephalic lateral slits or olfactory organs are large and deep. Mouth unusually large, oblong or oval, rather far back. Proboscis very long and slender; in section showing decussated muscular layers medially, above and below.

Anal papilla or cirrus often long and slender, delicate, contractile, often wanting owing to injury. It contains a continuation of the muscular layers of the body-wall.

Hubrecht has united *Micrura* to this genus, and in his report on the Nemerteans of the Challenger Exp. he proposes also to unite *Lineus* with it.

Such a wholesale massing together of these groups seems to me unnecessary and undesirable, and is, apparently, only thought of because of the difficulty of distinguishing the generic position of alco-

holic specimens—a difficulty that holds with quite as much force in many other groups of animals, which lose many of their characters by preservation in any known medium.

However, it seems to me that some of the large species referred to *Micrura* by authors really belong to *Cerebratulus*, especially those like *M. fusca* McIntosh (*non* Fabr. sp.)

The differences noted by McIntosh in the muscular layers of the proboscis appears to me of less importance than the special muscular structure of the body-wall which enables the species of *Cerebratulus* to swim actively at the surface, while the more slender and rounded species belonging to *Micrura* (restr.) and *Lineus*, so far as I have observed, are unable to swim, and do not voluntarily leave the bottom.

The broad, flattened form of the body with thin margins is the external expression of the internal musculature, adapting it to the undulatory swimming motion.

### Cerebratulus lacteus (Leidy) Verrill.

*Meckelia fragilis* Girard, Nord Amer. Monatsb., 1851 (*non* Goodsir, sp.)

*Meckelia lactea* Leidy, Proceedings Academy Natural Sciences of Philadelphia, vol. v, p. 243, 1851, (young); Verrill, Invertebrate Animals of Vineyard Sound, p. 336 [630], 1873, (young), *non C. lacteus* Hubrecht, Mont. sp.=*Lineus lacteus* McIntosh.

?*Meckelia Lizziæ* Girard, Proc. Acad. Nat. Sci. Philad., vol. vi, p. 366, 1854.

*Meckelia ingens* Leidy, Marine Invertebrate Fauna of Rhode Island and New Jersey, p. 11 (143), 1855; Verrill, Invertebrate Animals of Vineyard Sound, p. 336 [630], Plate XIX, figures 96, 96a.

PLATE XXXV, FIGURES 1, 1*a*; PLATE XXXVI, FIGURE 2; PLATE XXXVII, FIGURES 1, 1*a*, 1*b*; PLATE XXXIX, FIGURES 19, 20, 21.

Body flat, large and very long when full grown, sometimes becoming fifteen to twenty feet long and upwards of an inch in breadth, very contractile and changeable in length, breadth, and form. While swimming the body is turned up edgewise and thrown into many undulations and the motion resembles that of an eel, but is less rapid. The anterior part of the body for some distance back of the head is, in usual extension, narrower and thicker than the rest, with the margins rounded; the body then expands rather rapidly in breadth and at the same time becomes more flattened while the margins become thin and pale, and throughout the rest of its length the body continues thin and flat, gradually decreasing in breadth and thickness toward the posterior end, which is usually obtuse, or slightly emarginate, but occasionally, or when perfect, terminates in

a slender anal papilla. The posterior end is, however, seldom seen entire, owing to its extreme fragility and its tendency to disrupt itself when irritated. When disturbed the middle region of the body often contracts, while the anterior becomes thick and swollen.

The increase in breadth of the body and enlargement of the marginal regions marks the commencement of the crowded lateral lobes of the stomach and the genital organs, which can usually be seen through the translucent integuments; the cæcal lobes of the intestine usually appear as closely arranged, transverse, oblong spots, forming a regular row along each side, from their commencement nearly to the posterior end of the body, and usually having a slightly darker or more brownish tint than the central and marginal regions. The cæcal appendages, when seen from above or below, usually appear as simple, narrow, but often forked, and closely arranged lobes, but when examined in sagittal sections they are mostly lobed and forked horizontally. The genital organs are closely crowded between the cæcal pouches of the stomach, distally.

The head is exceedingly changeable in shape, according to its state of contraction or expansion, but is usually narrower and thinner than the adjacent part of the body. In full extension it is usually broad spear-shaped or rhomboidal, and more or less pointed at the apex, while marked lateral constrictions separate it posteriorly from the body, but in another moment it may contract to a broad rounded form, or it may even become deeply emarginate in front, with rounded lateral lobes, or it may change to a very narrow and elongated form with a sharp point. Ocelli are wanting.

The lateral cephalic slits are large and deep, extending the entire length of the head, and running forward close to and a little above the proboscis-pore, those of opposite sides not uniting together except by a very shallow furrow; they do not join the proboscis-pore, so that the snout is not four-lobed at tip, as in some allied species. Their margins are thin and mobile, often undulated or curled back so as to open the slits widely and expose the deep posterior pits, which, in life, are dull red within. Proboscis-pore large, terminal or subterminal.

Mouth very large, but variable in form as the head varies in shape, most frequently appearing as a long, narrow oval or oblong slit, its anterior end opposite the posterior ends of the lateral slits.

Proboscis exceedingly long, slender, round, whitish, and nearly smooth. When the worm is placed in alcohol or other irritating fluid the proboscis is usually ejected entirely without eversion (Pl.

xxxv, fig. 1*a*); in large specimens it is four feet or more long, and 3 or 4$^{mm}$ in diameter at the large end.

Color of small and moderate-sized specimens is translucent milk-white, cream-color, pale flesh-color, and occasionally pale salmon or pale pink, with the margins paler and more translucent; larger individuals are generally deeper flesh-color, cream-color, light salmon or ocher-yellow, and occasionally dull gray; the cæcal appendages of the intestine and the reproductive organs appear as a more opaque yellowish or pale brownish band along each side, near the pale margins; the lateral nerve-trunks are reddish.

Length of ordinary adult specimens, in extension, 500 to 1200$^{mm}$; breadth in middle 15 to 22$^{mm}$; some specimens are more than double these sizes.

Common, burrowing both in sand and mud at and above low-water mark, and in shallow water down to several fathoms in depth, from Florida to Massachusetts Bay, and locally found on the coast of Maine.

It is particularly abundant near low-water mark on the sheltered sandy shores of the New Jersey estuaries; Long Island Sound; Buzzard's Bay; Vineyard Sound; Cape Cod; and at Annisquam, Mass., north of Cape Ann. I have taken a number of well grown examples at Quahog Bay, on the coast of Maine, where it is associated with a colony of other southern species. I have not found it in the Bay of Fundy, where it is replaced by a closely allied arctic species (*C. fuscus*). Its southern range is not well determined, but I have seen specimens from Fort Macon, North Carolina, and others from St. Augustine, Fla., and Charleston, S. C., (W. R. Coe).

The largest specimen hitherto obtained I personally dug from the sand at low-water mark at Great Egg Harbor, N. J., April, 1872. This one, when extended, was 22 feet long and nearly an inch in breadth, in the middle. It could contract, however, to less than 6 feet in length, becoming, at the same time, much broader, thicker, and firmer. This gigantic specimen is, apparently, the most bulky nemertean that has ever been described, though species of *Lineus* far exceed it in length.

When preserved in alcohol it contracts very firmly and shows very plainly the contrast between the form of the anterior and middle regions of the body, the latter being decidedly flat with thinner margins. The head takes various shapes.

In alcoholic specimens the mouth is usually large and open. Sometimes numerous small whitish papillæ, probably containing the

genital pores, can be seen a short distance from the margin, both above and below; sometimes there are several in each transverse row; at other times only two or three are visible.

In transverse sections the great thickening of the interior longitudinal muscular layer in the marginal areas is strongly marked. (Pl. XXXIX, figure 19.)

The earliest name of this species that can be retained is apparently *C. lacteus* (Leidy), which was given to the white specimens that I now regard as the young of this species. I have adopted this name for the species, notwithstanding that *Lineus lacteus* (Mont.) McIntosh is now referred to *Cerebratulus* by Hubrecht. That the latter belongs to *Cerebratulus*, as here defined, I do not think possible.

A large species (*C. Pocohontas*) from Charelston, S. C., very briefly described by Girard under the name of *Meckelia Pocohontas*,* appears to be very similar to our species in size (3 feet long), form, and color, but he states that the snout is split vertically [by the proboscispore], indicating that the cephalic slits join the proboscis-pore in front, so that the snout, as he states, is four-lobed at the tip, which is not the case in our species. *C. Lizziæ*, from the same place, described in few words, at the same time (op. cit. p. 367), agrees with our species in respect to the color, snout, and slits, and may be identical with it.

*C. striolenta* (*Leodes striolenta* Girard, loc. cit.) also from Charleston, appears to be a typical *Cerebratulus*, but it is a very distinctly marked species, having a pink body, longitudinally striped, and with dark longitudinal blotches on the head; margins pale; length six inches; no ocelli.

### Cerebratulus Leidyi Verrill.

*Meckelia rosea* Leidy, Proc. Acad. Nat. Sci. Philad., vol. v, p. 244, 1851; Verrill, Invert. of Vineyard Sound, etc., pp. 336, [630] 1873.

? *Renieria rubra* Girard, Proc. Acad. Nat. Sci. Philad., vol. vi, p. 366, 1854.

*Cerebratulus roseus* Verrill, Check List Invert., p. 12, 1879, (*non C. roseus* (D. Ch.) Hubrecht).

PLATE XXXVIII, FIGURES 2, 2*a*.

Body elongated, rather slender in extension, rounded in the œsophageal region, decidedly flattened and wider farther back, but not so much so as in *C. lacteus* and allied species, nor do the margins become so broad and thin. Caudal papilla of moderate length, slender, white, often absent, owing to injury.

* Proc. Acad. Nat. Sciences, Philad., vol. vi, p. 366, 1854.

Head versatile, in extension decidedly long and narrow, often narrower than the body, regularly tapered to the acute tip.

Mouth large, elongated, with slightly crenulated white lips; its anterior end is about opposite the posterior ends of the lateral slits.

Lateral cephalic slits long and deep, with thin, translucent margins, often curved back so as to show the large interior cavity; in front they run very close to the proboscis-pore, which, in contraction, appears as a sub-terminal vertical slit. Proboscis very long and slender, pale pink in color. No ocelli. Cephalic ganglions large, showing through the integument as dark red spots.

Color of body dull red, or rose-color, or pale purplish, somewhat lighter beneath; usually with a lighter colored median line, and a red spot in the head corresponding to the ganglions; front of head and mouth area whitish; the closely arranged cæcal lobes of the intestine often show through the integument, especially beneath, as a pale brown band along each side. These cæcal appendages are numerous, and many of them are divided into two or three lobes distally.

Very common, burrowing in sand near low-water mark, from New Jersey to Cape Ann, Mass. It is abundant near New Haven, Conn.; Thimble Islands and Noank, Conn.; Newport, R. I.; and Wood's Holl, Mass.

This is a more strictly littoral species than the preceding. It seldom occurs much below low-water mark. The mucus that it secretes is more tenacious than that of most species, so that captive specimens often cover themselves quickly with adherent sand.

This species is generally found associated with *C. lacteus*, from which it can easily be distinguished by its decidedly red color, and its narrower and more slender body, without the very thin margins. It is also a more sluggish species and seldom swims freely. It is prone to break itself in fragments when captured.

It is unfortunate that the name *roseus*, which applies so well to this species, cannot be retained on account of the much earlier named Mediterranean species. I have, therefore, given it a new name in honor of Professor Leidy, who first described it.

It is quite probable, however, that *C. rubra* (=*Renieria rubra* Girard, op. cit., p. 366, 1854) is identical with this species. Girard's description is too brief and indefinite to determine this question. He describes *C. rubra* as uniform brick-red, paler beneath, and as lacking eyes; length 5 to 6 inches. Its form was nearly as in the present species. It was from Charleston, S. C., on sand-flats at Fort Johnson.

**Cerebratulus fuscus** (Fabr.) Verrill.

*Planaria fusca* Fabr., Fauna Grönlandica, p. 324, 1780.

*Meckelia olivacea* Rathke, Beitrage zur Fauna Norwegens, p. 324, 1843 (from Acta Akad. Cæs. Leop. Carol. Nat. Cur., vol. xx, 1843).

*Serpentaria fragilis* Goodsir, Ann. Nat. Hist., vol. xv, p. 387, pl. 20, figs. 1 and 2 1845.

*Meckelia serpentaria* Diesing, Systema Helm., vol. i, p. 266, 1850.

*Gordius fragilis* Dalyell, Pow. Creat., vol. ii, p. 55, pls. 6, 7, and 7 (*bis*), 1853.

*Meckelia serpentaria* Leuckart, Archiv. fur Naturges., ii, p. 187, 1859.

*Serpentaria fragilis* Johnston, Catalogue Brit. Mus., p 28, 1865.

*Cerebratulus angulatus* McIntosh, British Annelids, part i, Nemerteans, p. 195, 1873.

*Cerebratulus* (?) sp. undetor. (*a*) Verrill, Report on Invert. Anim. of Vineyard Sound, p. 336 [630], 1874.

*Cerebratulus fragilis* (?) Jensen, op. cit., p. 85, 1878.

*Cerebratulus grandis* (Sars) Jensen, op. cit., p. 97, pl. 8, figs. 17–22.

*Cerebratulus fuscescens* Levinsen, Bidrag til kundskab om Grönlands Turbellariefauna, p. 40 [202], 1879.

PLATE XXXVII, FIGURES 2 TO 2*c*.

Body large, stout, rounded for a considerable distance back of the head, and thence broad and much flattened to the posterior end, the edges thin and usually pale in color. Head very changeable in form, often broad lance-shaped, with acute snout, changing quickly to ovate, rounded, or even emarginate forms. Ocelli wanting. Mouth large, oblong. Cephalic slits large and deep, moderately long; they do not meet in front, nor run into the proboscis-pore, but lie in a higher plane Anal cirrus slender, easily detached.

Color, above, dull ash-gray, greenish gray, slate-color, clay-color, grayish olive, or dirty brown, paler below, and with paler margins, within which, on each side, a red line, showing through the integument, marks the position of the large lateral nerves. Sometimes the back is mottled with lighter, and darker gray or slate; mouth surrounded by white, reddish at the anterior angle.

Length up to two feet or more. A specimen taken at Todd's Head, Eastport, Me., under stones at low-water, Aug. 19, 1870, measured $400^{mm}$ in length, when moderately extended; breadth, in middle, 12 to $14^{mm}$, but it could contract to less than $100^{mm}$ in length.

This is a northern and arctic species. I have taken it at Halifax, N. S.; Grand Menan, N. B.; Eastport, Me., under stones and in sand and gravel near low water mark, and beyond in shallow water to 20 fathoms or more. South of Cape Cod it occurs in 15 to 45 fathoms on bottoms of sand and mud in the cold areas swept by the

arctic current, as off Gay Head, in 19 fathoms, and off Block Island, in 29 fathoms. It is also found on the coasts of Greenland, northern Europe, and Scotland.

This species usually lives in burrows under stones, in muddy or sandy places, at and below low-water mark, but when disturbed it swims readily and rapidly with vigorous eel-like undulations of the posterior flattened portion of its body, which is carried with the greater diameter vertical while swimming. In this habit it agrees with *C. lacteus* and several other large species, but it is, perhaps, more active and more vigorous than *C. lacteus*, and somewhat less liable to disrupt its body when captured. Like *C. lacteus* it is occasionally taken at night in surface nets, showing that it is nocturnal in its habits and voluntarily leaves its burrows and swims free at the surface.

After long preservation in alcohol the slate-color of the body and the white margins are often distinctly visible. In some alcoholic specimens the small and slender anal papilla is still preserved, but it is so fragile that it is generally lost during capture or in the violent contractions caused by the alcohol.

Our species is probably identical with the European species named *C. angulatus* by McIntosh, who supposed his species to be the *Planaria angulata* of Fabricius (Fauna Grönlandica). The latter is, however, our *Amphiporus angulatus*, as stated on a former page.

Formerly* I supposed that the Greenland species named *Planaria fusca* by Fabricius might be the brown variety of *Lineus viridis*, but a more careful study of his description, in which the absence of ocelli, the presence of lateral cephalic slits, the rounded form of the anterior, and the distinctly flattened form of the posterior part of the body are mentioned, has convinced me that the species he had in hand was the common dark-colored, large, northern *Cerebratulus*, which has received many later names. His statement that it lives in sand on the shores confirms this view. Moreover, this same *Cerebratulus* has been recently recorded from the Greenland coast and referred to the Fabrician species by Levinsen, as quoted above. He, however, adopts the later emended form of the name, quite unnecessarily it seems to me. Hence I have restored the original name, first given by Fabricius to this species.

* Proc. U. S. Nat. Mus., vol. ii, p. 185, 1879.

**Cerebratulus luridus** Verrill.

*Meckelia lurida* Verrill, Report on Invert. of Vineyard Sound, etc., p. 336 [630] 1873.

*Cerebratulus luridus* Verrill, Check List Invert., p. 12, 1879.

PLATE XXXVI, FIGURE 3; PLATE XXXVII, FIGURE 3.

Body large, rather stout, very changeable in form, broad, flat, thin posteriorly, where the lateral cæca and reproductive organs are developed; these diminish anteriorly and do not extend forward into the narrower, rounder, and thicker portion which occupies nearly one-fourth the whole length. Head very changeable, often separated from the body by a constriction; in expansion often spade-shaped, obtuse, or pointed. Lateral cephalic slits very long and deep; in front they are connected together by a shallow furrow, above the proboscis-pore. Mouth large, usually in the form of a long slot, commencing about opposite the posterior end of the lateral slits. Proboscis long and slender. Caudal papilla small, slender, acute.

Color reddish brown to dark olive-brown, chocolate-color, or purplish brown, darkest anteriorly, and with pale margins; the cæcal lobes of the intestine show through the integument as dull brownish or ocher-yellow transverse bars; usually there is a brown or reddish median dorsal line, and a pale ventral line. Some dark specimens are marked with several narrow lighter reddish or purplish longitudinal lines. Young specimens are usually reddish brown or liver-brown with paler snouts.

Length 150 to 250$^{mm}$; breadth 8 to 12$^{mm}$. Described from life. (No. 723).

Off Gay Head, 19 fathoms, soft mud, 1871; off Buzzard's Bay, 25 fathoms; and off Block Island, 29 fathoms, sandy mud, 1871; Casco Bay, 10 to 68 fathoms, 1873; Massachusetts Bay, in many localities, 1877, 1878, 1879, in 10 to 100 fathoms; Bay of Fundy; off Halifax, N. S., etc., common; off Martha's Vineyard, 192 fathoms, 1883. Numerous specimens of various sizes from 1 to 8 inches long were taken in Cape Cod Bay, in 15 to 21 fathoms, soft mud, Aug. 29, 1879. The larger ones were filled with eggs.

DOUBTFUL SPECIES.

**Cerebratulus medullatus** Hubrecht, Voyage of the Challenger, vol. xix, pp. 39, 50, pl. xi, fig. 10; pl. xii, figs. 9, 10, 1878.

PLATE XXXIX, FIGURE 17.

This species was described from a mere fragment, without head or tail. It is said, however, to differ from other species in the structure of the body-wall, which is thinner than usual.

The inner glandular layer of the integument and the inner basement membrane are wanting, as distinct layers, so that the outer glandular layer and its basement layer are in contact with the outer-longitudinal muscular layer. The median dorsal nerve, or nervous thickening, is also unusually large and distinct, being from one-third to one-fourth as thick as the core of the lateral nerve-trunks.

Off Nova Scotia, in 85 fathoms.

This species is probably not a *Cerebratulus*, as here defined, but more likely belongs to *Lineus* or *Micrura*, and perhaps to some of the species described above.

## Suborder II, GYMNOCEPHALA.

*Holocephala* Diesing, 1850, *non* Müll., 1835.
*Gymnocephalidæ* Kefferstein, Zeitsch. für wiss. Zool., xii, 1862.
*Anopla* (*pars*) McIntosh, Nemerteans, p. 203.
*Palæonemertini* Hubrecht; Carus.
*Palæonemertea* Hubrecht, Voy. Challenger, xix, p. 5, 1887.
*Palæomertina* Lang, Text-Book of Comparative Anat., p. 178, 1891.

Head without lateral slits, but sometimes having shallow transverse or oblique fossæ connected with small, ciliated (olfactory) pouches or ducts leading to the posterior ganglions; sometimes destitute of both fossæ and ciliated ducts. Mouth distinct, situated back of the ganglions.

Proboscis long and slender, more simple in structure than in the Rhagadocephala. Usually only two (lateral) longitudinal blood vessels are present.

Ocelli often numerous, variously arranged, sometimes wanting.

Lateral nerve trunks sometimes situated between the basal layer of the cutis and the external circular muscular layer; sometimes outside of the longitudinal muscular layer; and sometimes in the midst of the muscular layer of the body-wall; usually connected with a continuous nervous plexus.

In the classification adopted by Hubrecht and several other writers this group and the Rhagadocephala (or *Schizonertina*) are both raised to the same rank as the *Enopla*. To me they appear to be of subordinate value, as here indicated.

The species are all marine, and, so far as known, none of them undergo a marked metamorphosis.

Family, CEPHALOTHRICIDÆ McIntosh, Nemerteans, p. 208.

Body slender. Head elongated. Superior ganglions and commissure situated decidedly in front of inferior ones. Cephalic fossa and pits wanting. Ocelli usually few or absent; sometimes numerous. Two longitudinal blood vessels.

**Cephalothrix** Œrsted, Kroyer's Tidss., iv, p. 573, 1844.

*Astemma* Œrtsted, Kroyer's Tidss., iv, p. 574, 1844 (t. McIntosh).

Body slender, terete or nearly so. Head terete, much elongated, tapering to a point in extension. Mouth small, situated far back.

**Cephalothrix linearis** (Rathke) Œrsted.

*Planaria linearis* Jens Rathke, Skrivter af Naturhist. Selsk., vol. v, p. 84, tab. [illegible] fig. 11, 1799.

*Planaria filiformis* Johnston, Zool. Jour., vol. iv, p. 56, 1829 (t. McIntosh).

*Nemertes* (*Borlasia*) *rufifrons* Johnston, Mag. Zool. and Bot., vol. i, p. 538, pl. XVII, figs. 4 and 5, 1837 (t. McIntosh).

*Cephalothrix linearis* Œrsted, Entw. Plattw., p. 82 (note under *C. cæca*), 1844 (t. McIntosh).

*Cephalothrix bioculata* Œrsted, Kroyer's Nat. Tidss., vol. iv, p. 573, 1844 (t. McIntosh).

*Astemma filiformis* Johnston, Catalogue Brit. Mus., p. 19, 1865.

*Cephalothrix filiformis* McIntosh, Rept. Brit. Assoc., 1867, Trans. Sect., p. 92, 1867.

*Cephalothrix linearis* McIntosh, British Annelids, Part I, Nemerteans, p. 208, pl. IV, figs. 4 and 5; pl. XVIII, fig. 15; pl. XXI, figs. 2, 8, 13; pl. XXIII, figs. 12 to 16, 1873.

PLATE XXXVI, FIGURES 4, 5; PLATE XXXIX, FIGURES 10 to 13, 14, 15.

Body small, nearly terete, changeable; in extension very slender, elongated, often linear or hair-like, frequently coiled in a close spiral form, usually rather thickest in the middle and tapered both ways, but often with the posterior end thicker and obtuse. Head very long and round, in full extension tapering to a slender sharp tip, in contraction often circularly wrinkled; usually, in mature specimens, without distinct ocelli; sometimes dark specks of pigment, irregularly arranged, resemble imperfect ocelli. Young examples usually

have a pair of distinct ocelli. No cephalic slits nor fossæ. Mouth small, situated far back from the snout; and usually with slightly prominent lips.

Color pale yellow, flesh-color, or cream-color, varying to pale salmon and greenish white, often with the anterior region deeper salmon or reddish, or with a median red line; sometimes the posterior part of body is bright salmon; the head and anterior portions of body often show a whitish or drab median line, due to the proboscis; lower surface paler than the upper. Proboscis very long and slender; when exerted, covered with slender acute papillæ.

Length 50 to $75^{mm}$; diameter ·5 to $1^{mm}$.

Long Island Sound to Nova Scotia, at many localities, between tides under stones and in sand. Noank, Conn.; Newport, R. I.; Wood's Holl, Mass.; Portland, Me.; Eastport, Me.; Halifax, N. S., etc. Also common on the northern coasts of Europe.

This species often occurs gregariously, many individuals being intricately coiled up together in a mass, often mingled with numerous pale young ones, of various sizes.

## Family, CARINELLIDÆ McIntosh.

Body elongated, roundish, decreasing backward. Head broader than body, obtuse anteriorly. Mouth small, not far back. Cephalic shallow fossæ and olfactory sacs present. Ocelli often wanting.

**Carinina** Hubrecht, Voy. Challenger, vol. xix. p. 5.

"Closely allied to *Carinella*, from which it differs in the presence of a distinct posterior brain lobe, situated with the rest of the brain and nerve-stems in the integument, outside of the body musculature. A ciliated canal penetrates into this posterior brain lobe."

**Carinina grata** Hubrecht, op. cit., pl. I, figs. 1–3; plates II, III, IV; pl. VI, figs. 1–3; pl. XI, figs. 1, 2.

This species is known only from two alcoholic specimens, which were very fully studied anatomically.

Off the East Coast of the U. S. States in 1240 and 1340 fathoms.

## Order III, BDELLOMORPHA.

Body short, stout, flattened, and leech-like in appearance, with a large rounded sucker or acetabulum at the posterior end, as in the leeches. Head indistinct. No ocelli. Anterior end emarginate, with neither lateral slits nor grooves. Mouth at the bottom of the anterior emargination.

Proboscis seldom protruded in captivity, small, slender, unarmed, but with a small special bulb and sac in the middle region; proboscis-pore close to the mouth, in the anterior notch.

Intestine not lobulated, slender, convoluted, longer than the body; anus at the base of the sucker. Reproductive organs voluminous, filling the larger part of the body. A median dorsal and two lateral blood-vessels, with numerous branches.

Muscular walls of the body consist of an external circular, and an internal longitudinal layer. Lateral nerve-trunks are not included in the muscular layers; they are united by a posterior commissure.

This singular group is united to the Enopla by some writers, mainly on account of the rudimentary bulb and sac in the proboscis, which certainly indicate some relationship. The simple, convoluted intestine and other peculiar features appear to me of ordinal value.

### Family, MALACOBDELLIDÆ Semper.

Characters not distinguishable from those of the sub-order.

### Malacobdella Blainville.

Dict. Sci. Nat., vol. xlvi, p. 270; Blanchard, Ann. des sci. nat., ser. 3, vol. iv, p. 364, 1845; op. cit., viii, p. 142, 1847; op. cit., vol. xii, pp. 267–276, pl. 5, 1849, anatomy.
*Phylline* Oken, 1815, (*non* Abilg, 1790).

This is the only genus of the order known. Therefore the generic characters are not distinguishable from those of the order.

### Malacobdella obesa Verrill.

Report on Invert. of Vineyard Sound, etc., pp. 458 [164] and 625 [331], pl. XVIII, fig. 90, 1873.

WOOD-CUT, No. 9.

Body stout, broad, thick, convex above, flat below, broadest near the posterior end, narrowing somewhat anteriorly; the front end is broadly rounded, with a median vertical slit, in which the mouth is situated. Acetabulum large, rounded, about as broad as the body. Intestine convoluted posteriorly, visible through the integument.

Between the intestine and lateral margins, especially posteriorly, the skin is covered with small stellate spots, looking like openings, around which are large numbers of small round reproductive vesicles. Color yellowish white. Length, 30 to 40$^{mm}$; breadth, 12 to 15$^{mm}$.

Whole coast of New England; abundant in Massachusetts Bay. Parasitic in the branchial cavity of the long clam (*Mya arenaria*).

This species is closely related to *M. grossa* of Europe, and may prove to be identical with it. The latter occurs mostly in *Mya truncata* and *Cyprina Islandica*.

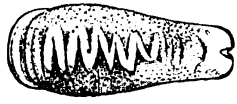

Fig. 9. *Malacobdella obesa*, dorsal view, nat. size.

**Malacobdella mercenaria** Verrill.

*Malacobdella grossa* Leidy, Proc. Acad. Nat. Sciences Philad., vol. v, p. 209 (*non* Blainville).

*Malacobdella mercenaria* Verrill, Report on Invert. of Vineyard Sound, etc., pp. 458 [164] and 625 [331], 1873.

PLATE XXXIX, FIGURE 20.

Body, in extension, elongated, oblong, with nearly parallel sides, or tapering slightly anteriorly; anterior end broad, obtusely rounded, emarginate in the center, but not deeply fissured. In contraction the body is broader posteriorly. Dorsal surface a little convex; lower side flat. Acetabulum round, rather small, about half the diameter of the body in the contracted state, but nearly as broad when the body is fully extended. The intestine shows through the integument distinctly; it is slender, and makes about seven turns or folds. Color pale yellow, with minute white specks beneath and on the upper surface anteriorly, giving it a hoary appearance; middle of the dorsal surface irregularly marked with flake-white; laterally reticulated with fine white lines.

Length in extension, 25$^{mm}$; breadth, 4$^{mm}$; in partial contraction, 18$^{mm}$ long; 5 to 6$^{mm}$ wide.

New Haven, parasitic in the branchial cavity of the round clam (*Venus mercenaria*), October, 1871. Philadelphia, in the same species of clam (Leidy).

## Addenda to the Enopla.

I take this opportunity to describe two very remarkable new forms of pelagic nemerteans, of which several specimens were taken by the U. S. Fish Commission Steamer Albatross, in the region of the Gulf Stream. Whether they occurred at the surface or near the bottom I am unable to say, but their form and structure is eminently adapted to a purely pelagic mode of life. In form they somewhat recall *Sagitta*, though they are much larger and stouter. The internal structure is, however, entirely nemertean, and not very different from that of the typical Enopla. In that group, however, they should form at least a distinct family (*Nectonemertidæ*). They also have some affinity with *Pelagonemertes*, but differ from that genus widely in form, as well as in having a distinct head and caudal fin. lateral cirriform organs in one species, etc. The latter, moreover, has long, much subdivided intestinal diverticula, which is not the case with our new genera. The resemblance in the structure of the muscular walls of the body and the nervous system is quite marked.

Several forms occur among the few specimens of *Nectonemertidæ* hitherto obtained. Some of them are entirely destitute of the lateral arms or cirri of the neck, which in others are large and long and give them a very striking appearance. But as small specimens of *Nectonemertes* occur in which the lateral cirri are of small size, it is probable that they would be entirely absent in still smaller specimens of that genus. In the second genus (*Hyalonemertes*) they are probably never developed.

Although I have prepared many microscopic sections of two specimens of *Nectonemertes* of different ages, I have not yet had sufficient opportunity to work out several important parts of their anatomy,—especially the structure of the brain and certain special organs in the head, supposed to be sensory. But since there is, at this time, no opportunity to illustrate the details of the anatomy, I propose to describe here only the more prominent features, reserving details for another occasion.

### Family, Nectonemertidæ Verrill.

Body with highly muscular striated walls, adapted for swimming actively, elongated, more or less flattened, and with a differentiated, muscular caudal fin; the dorsal and ventral surfaces are similar.

Proboscis with a distinct bulb and sac. Mouth far forward, close to the proboscis-pore. Intestine straight, with large lateral pouches,

which are often bilobed; anus at the posterior end of the caudal fin. Lateral nerves large, not included in the muscular walls of the body, united posteriorly. A median dorsal, and two lateral blood-vessels are well developed.

Muscular walls of the body are composed mainly of a thin, outer, circular layer and a thicker inner, longitudinal muscular layer, in which the fibers are arranged in distinct bundles, except in the thinner marginal regions. A pair of long, muscular, cirriform appendages is developed from the sides of the nuchal region in one genus.

## Nectonemertes, gen. nov.

Body decidedly flattened and with thin borders along the sides; caudal fin usually broadest at the end and sometimes bilobed. Head separated from the body by a more or less distinct neck-like portion. Lateral cirriform appendages project from the neck or posterior part of the head, in the adult. Mouth near the front of the head, just below the terminal proboscis-pore.

Proboscis long, slender, with a small bulb and sac; its sheath extends nearly to the posterior end of body. Lateral lobes of the intestine exist nearly to the end of the intestine, even into the caudal fin.

Special sense organs,* imbedded in the integument of the lower side of the head, form a cluster on each side, their ends projecting as small papillæ. Eyes of the ordinary type are, apparently, wanting. Probably the species are transparent in life and swim actively, like *Sagitta.*

### Nectonemertes mirabilis Verrill, sp. nov.

PLATE XXXVIII, FIGURE 1.

*Description of the adult:* Size large, up to 2 inches or more in length. Body rather elongated, decidedly flattened and with abruptly thinner marginal regions, smooth, with the walls somewhat translucent, longitudinally and transversely striated, elastic; in the middle region of the body the sides are nearly parallel; posteriorly it narrows rather rapidly to the base of the tail, and at this place, in some examples, the thin margin of the body forms a sort of fin or thin rounded lobe on each side.

* The precise nature of these organs has not been ascertained, but they are probably special sense organs.

The tail gradually thins out to the end and at the same time increases in width by the development of the thin marginal regions, thus forming a true caudal fin, in form somewhat like that of a fish. Its posterior margin is emarginate in the largest specimens, with a distinct notch in the middle, where the anus is situated, but in other specimens it is truncate. The integument of the tail shows strong longitudinal muscular fibers toward its base, while the edges are thin and delicate.

The head is ovate in form, narrowest, but obtuse, in front, considerably flattened, and usually separated from the body by a distinctly narrower neck. From the back part of the head, or commencement of the neck, a long, tapering cirrus arises on each side. The cirri have a thick, roundish, muscular base from which they taper gradually to the long, slender, lash-like, often coiled tip. These organs seem to be mere extensions of the muscular walls of the body and are not hollow.

On the ventral surface of the head and occupying a large ovate patch on each side, there is a group of small acute papillæ, projecting slightly above the surface; they are arranged in three or four irregular rows, and are connected beneath the integument with pyriform organs which can be seen by transmitted light as opaque yellowish bodies.

The proboscis-sheath is well developed and extends back nearly to the base of the tail, where it is abruptly narrowed to a short muscular band that joins the wall of the body. The proboscis is long and slender, with a small rounded muscular bulb* and a small saccular organ, much as in ordinary Enopla, though relatively smaller. When the proboscis is partially protruded, as is the case in one example, it is somewhat clavate distally and is covered with small papillæ. In transverse sections its structure is similar to that of the typical Enopla; its internal glandular layer is thick.

The intestine is large and straight; its lateral pouches are large, not much elongated, mostly bilobed distally, those in the tail becoming small and simple. The generative organs, in the form of rather large, round or ovate vesicles, occupy the lateral and ventral regions of the body-cavity, between and beyond the intestinal pouches.

In transverse sections the walls of the body are rather thin; the outer layer of circular muscular fibers is thinner than the inner

* I have been unable to find any armature in the only specimen hitherto prepared for this purpose, but the stylets, if they existed, may have been destroyed by the acidity of the alcohol in which it was preserved.

layer, which is made up of longitudinal fibers arranged in bundles, so that its inner surface, in the sections, is strongly crenulated, or deeply furrowed; from the indentations between these bundles numerous strong vertical bands of muscular fibers extend from the dorsal to the ventral body-walls, between the internal organs. Toward the margins the muscular layers thin out rather abruptly, leaving the marginal portions thin and without longitudinal bundles. The general structure of the interior of the body-cavity is loose, with many spaces in the porous parenchyma, which is feebly developed, as compared with that of other nemerteans.

The lateral nerve-trunks are very large and quite interior to the muscular layers. They are situated ventrally, some distance from the edges, and near the commencement of the thin-walled marginal portion of the body. In transverse sections they are elliptical or rounded, with an excentric translucent fibrous core along the dorsal side, thus giving the cellular portion a thick-lunate or reniform shape. The lateral nerves are large and conspicuous even back to the caudal fin, where those of opposite sides unite.

The median dorsal blood-vessel and the two lateral blood-vessels are well developed and situated nearly as in typical Enopla. The lateral blood-vessels are subventral and only a short distance interior to the nerve-trunks.

There are no memoranda as to the color of the living specimens. All had been placed in alcohol when first seen by me. One that had been in alcohol only a short time was distinctly salmon, or pale orange, in tint; the others had lost all color, if they had any when living. They may have been white or colorless, and were doubtless translucent, like many other pelagic creatures. Even in alcohol some of them show considerable translucency,—nearly as much as the larger species of *Sagitta*.

The largest specimens, when first examined by me, were about 2·5 inches long and ·50 wide; subsequently they have contracted considerably by long preservation in strong alcohol.

The largest specimen now measures as follows: length $38^{mm}$; breadth of body $9^{mm}$; vertical diameter of body $2^{mm}$; length of cirri $14^{mm}$; length of head $4^{mm}$; breadth of head $6^{mm}$; breadth of caudal fin $4^{mm}$.

*Descriptions of immature specimens:* A specimen from station 2076 is smaller and more slender than those described above. It has a narrower head and shows scarcely any constriction at the neck. The caudal fin is somewhat elliptical, being widest in the middle and

truncate at the end. Otherwise it agrees very well with the larger specimens. The cirri are, however, relatively shorter, their length being scarcely more than the breadth of the body, but they taper to slender tips, as do those of the adults. They are directed backward.

This specimen is somewhat translucent in alcohol and the thin, marginal bands are very distinct along the sides of the body and in the tail fin. The intestinal pouches, proboscis-sheath, and other internal organs show more or less distinctly, especially posteriorly. In the head there are about 20 sense organs (?) in each lateral cluster.

Length $35^{mm}$; breadth of body $5^{mm}$; length of head to base of cirri $4^{mm}$; its breadth $4^{mm}$; length of cirri $5^{mm}$.

Perhaps this may be a male and the larger and flatter specimens females.

A specimen from station 2229 agrees in most respects with the fullgrown ones described above, except that it is smaller and has short nuchal cirri. In this the body is relatively narrower and less flattened than in the larger examples, but the head, caudal fin, and proboscis are nearly as described and figured. The nuchal cirri are, however, short, tapered, blunt, not much longer than half the breadth of the head, and stand out rather rigidly from the sides of the neck, and nearly at right angles with it.

This specimen is about $30^{mm}$ long; $7^{mm}$ broad; caudal fin $3{\cdot}5^{mm}$ broad; length of cirri $2^{mm}$. It has been treated with hardening reagents for sections, and is therefore strongly contracted.

A single specimen was taken at each of the following stations by the steamer Albatross:

Station 2036, N. lat. 38° 52′ 40″, W. long. 69° 24′ 40″, 1735 fathoms. Adult.

Station 2076, N. lat. 41° 13′ 00″, W. long. 66° 00′ 50″, 906 fathoms. Young with small cirri.

Station 2229, N. lat. 37° 38′ 40″, W. long. 73° 16′ 30″, 1423 fathoms. Young with small cirri.

Station 2236, N. lat. 39° 11′ 00″, W. long. 72° 08′ 30″, 636 fathoms. Adult.

The specimen from Station 2236 is marked as having been taken in the trawl-wings. Many of the specimens of other groups, thus taken, undoubtedly came from near the bottom, but on the other hand, it is easy for any surface species to be taken in the same nets while the trawl is being lowered or when it is being taken in. Con-

cerning the other specimens there are no memoranda, but from their good condition it is more probable that they were all taken in the trawl-wings than in the trawl itself.

### Hyalonemertes, gen. nov.

Body elongated, fusiform, somewhat flattened, having no evident constriction at the neck, nor marked marginal folds, except in the caudal fin. Cirri wanting. Head not differentiated from the neck. Caudal fin well developed.

Proboscis long and slender, with a distinct bulb and sac, and, apparently, having a small central stylet. Lateral pouches of the intestine numerous, short, not much divided. Walls of the body thicker and more gelatinous than in *Nectonemertes*, not showing transverse striations, but covered with fine granulations; inner muscular layer longitudinally striated.

Pyriform bodies not present in the head. Eyes apparently wanting. Neither ciliated grooves nor pits were noticed on the head.

### Hyalonemertes Atlantica, sp. nov.

Body of the larger specimen moderately flattened, fusiform, about four times longer than broad, gradually tapered both ways; head blunt, flattened; caudal fin short, stout at base, a little broader toward the end, which is thin and slightly emarginate. Along the sides of the body the marginal fold is very narrow and indistinct, the edges being rounded; the folds become more evident posteriorly and form the borders of the caudal fin.

The integument appears somewhat soft and gelatinous, and is more translucent than in *Nectonemertes*, and not so firm. The whole surface is covered with minute soft granules hardly visible to the naked eye, but appearing, when magnified, something like fine shagreen; beneath the surface the longitudinal muscular striations can be seen. The granulation of the surface is finer and less distinct on the head. The proboscis is not protuded in this specimen. The small mouth is just below the end of the snout; near the upper margin there is a small round papilla.

Length of the larger specimen, from Station 2724, $38^{mm}$; breadth of body $11^{mm}$; breadth of caudal fin $6^{mm}$.

Length of the smaller specimen, from Station 2428, $20^{mm}$; breadth $3{\cdot}5^{mm}$.

The smaller specimen, just mentioned, is rather more slender than the larger one; its caudal fin is distinctly bilobed, with the lobes

well rounded at the end. The long slender proboscis is partially protruded, so as to show the bulb and sac in the exserted part, but not at the end; there appears to be a small stylet, but the mounted specimen is not sufficiently transparent to show its form; the exterior of the exsert proboscis is finely papillose. The large proboscis-sac extends back to about the posterior fourth; it is abruptly narrowed near the posterior end, and a band of muscular fibers near the end, on each side, binds it to the body wall. A single specimen was taken by the Albatross at each of the following stations:

Station 2428, N. lat. 42° 48′, W. long. 50° 55′ 30″, in 826 fathoms. Young.

Station 2724, N. lat. 36° 47′, W. long. 73° 25′ 00″, in 1641 fathoms. Adult.

## EXPLANATION OF PLATES.

### Plate XXXIII.

Figure 1.—*Amphiporus angulatus.* Dorsal view with the proboscis partially protruded, natural size; 1*a*, the same, ventral view of the head and anterior part of the body. Eastport, Me., low-water, Aug. 7, 1872.

Figure 2.—The same. Dorsal view of a specimen of the reddish brown variety, more enlarged. Massachusetts Bay.

Figure 3.—*Amphiporus multisorus*, sp. nov. Dorsal view of the head and anterior portion of the body; × 2. Eastport, Me.

Figure 4.—*Amphiporus virescens* V. Dorsal view; × 3. Noank, Conn., July 24, 1874; 4*a*, the same specimen, posterior end, more enlarged; 4*b*, the same, ventral view of the head, more enlarged; 4*c*, the same, dorsal view, more enlarged; 4*d*, the same, head with the slightly protruded proboscis; × 8; 4*e*, the same, nearly profile view of the head; × 8. Wood's Holl, Mass., July 13, 1875.

Figure 5.—*Amphiporus ochraceus* V. Dorsal view; × 4; 5*a*, the same, central stylet of the proboscis, much enlarged. Wood's Holl.

Figure 6.—The same. Head and anterior portion of another specimen more contracted; × 6. Eel Pond, Wood's Holl, July 19, 1875.

Figure 7.—*Amphiporus cruentatus* V. Dorsal view; × 3. Noank, Conn., July 14, 1874 (No. 740).

Figure 8.—The same. Dorsal view of a larger specimen; × 6; 8*a*, head of the same specimen, more enlarged. Wood's Holl.

Figure 9.—*Tetrastemma candidum.* Dorsal view of a greenish specimen; × 6.

Figure 10.—The same. Dorsal view of a specimen of the yellow variety; somewhat compressed under the microscope; × 3, low-water; 10*a*, the same specimen, showing variation in the form of the head owing to the different degree of extension. Casco Bay, low-water, 1873.

Figure 11.—*Tetrastemma vermiculus*, var. Dorsal view; × 7; 11*a*, 11*b*, other views of the head of the same specimen in different states of contraction; 11*c*, proboscis-armature of the same, much enlarged. Wood's Holl, on piles of wharf, July 24, 1875.

Figures 1, 2, and 10 are by J. H. Emerton; figures 3, 4*a* to 4*e*, and 11 to 11*c*, are by the author; the rest are by J. H. Blake. All are from living specimens.

PLATE XXXIV.

Figure 1.—*Amphiporus frontalis*, sp. nov. Dorsal view; × 2; 1*a*, the same specimen, ventral view of the head. (No. 10) Eastport, Me., 86 fath., 1870; 1*b*, the same, dorsal view of the head of another specimen. This was pale salmon with pale purplish spots on the sides due to the ovaries; proboscis-sheath greenish. (No. 86) Eastport, Me., low-water.

Figure 2.—*Amphiporus cæcus*, sp. nov. Dorsal view; × 5; 2*a*, 2*b*, dorsal and ventral views of the head of another specimen (No. 721); 2*c*, extruded proboscis of the same specimen, enlarged.

Figure 3.—*Amphiporus bioculatus*. Dorsal view; × 5. Off Fisher's I., Conn., July 22, 1874. The ocelli are too much obscured by the color in printing.

Figure 4.—The same. Dorsal view of a younger specimen of the light colored variety, compressed under the microscope; × 10. Newport, R. I.

Figure 5.—*Amphiporus roseus*. Dorsal view; × 2; 5*a*, the same, head and anterior portion of body, dorsal view; × 4; 5*b*, the same, side view; × 4.

Figure 6.—*Amphiporus tetrasorus*, sp. nov. Dorsal view; × 3. Massachusetts Bay, 1878.

Figure 7.—*Amphiporus heterosorus*, sp. nov. Head and anterior portion of body, dorsal view; × 1½. Massachusetts Bay, 1878.

Figure 8.—*Amphiporus frontalis*, sp. nov. Dorsal view of a small specimen partly contracted; × 3. Off Witch Rock, Massachusetts Bay, September, 1877.

Figure 9.—*Amphiporus mesosorus*, sp. nov. Head and anterior portion of body, dorsal view; × 3. Massachusetts Bay, off Salem, August 13, 1877.

Figure 10.—*Tetrastemma elegans* V. Type specimen from life. Dorsal view; × 6.

Figure 11.—*Tetrastemma vermiculus*. Young, dorsal view; × 12.

Figure 12.—*Tetrastemma vermiculus*, var. *catenula*. Dorsal view; × 8. Noank, Conn.

Figure 13.—*Tetrastemma dorsale*. Dorsal view of head and anterior portion of body with protruded proboscis; × 8. Casco Bay, 1873.

Figure 14.—*Tetrastemma dorsale*, var. *marmoratum*. Dorsal view; × 3. The lighter and darker mottlings are not sufficiently distinct. Casco Bay.

Figure 15.—*Amphiporus bioculatus* (?). Very young, dorsal view; × 12; compressed under the microscope, while alive. Newport, R. I., September 1, 1880, station 851, 12½ fath. Color translucent white; eyes black.

Figure 16.—*Amphiporus* (*Nareda*) *superba*. Copy of the original figure.

Figure 17.—*Amphiporus heterosorus*, sp. nov. Head and anterior part of body, dorsal view; × 2.

Figures 2, 3, 10, 11 are by J. H. Blake; figures 4, 8, 13, 15 are by J. H. Emerton; the rest, except 16, are by the author. All are from living specimens.

PLATE XXXV.

Figure 1.—*Cerebratulus lacteus*. Young, natural size; 1*a*, the same, ventral view of head and extruded proboscis, natural size. New Haven, Conn.

Figure 2.—*Emplectonema giganteum* V. Dorsal view of a specimen not full grown; ½ natural size.

Figure 3.—*Amphiporus cruentatus* V. Dorsal view; × 4.

Figure 4.—*Amphiporus agilis* V. Dorsal view; × 4.

Figure 5.—*Amphiporus glutinosus* V. Dorsal view; × 2.

Figure 6.—*Tetrastemma vittatum* V. Dorsal view; × 8; compressed under the microscope, while living.

Figure 7.—The same. Dorsal view of the variety with a single pale dorsal stripe; [illegible].

Figure 8.—*Tetrastemma vermiculus.* Dorsal view; × 8. Wood's Holl, Mass., low-water.

Figure 9.—*Tetrastemma candidum* (?). Dorsal view of a very young specimen, much enlarged; compressed under the microscope, while living. Newport, R. I., sta. 851, 12½ fath., September 1, 1880.

Figure 10.—The same. Dorsal view of a somewhat older specimen. Savin Rock, Conn., October 18, 1887.

Figure 11.—*Tetrastemma vermiculus*, var. *catenula*; × 2.

Figures 1, 6, 8 are by J. H. Emerton; figures 3, 11, are by J. H. Blake; the rest are by the author. All are from living specimens.

PLATE XXXVI.

Figure 1.—*Micrura affinis.* Dorsal view; × 4. Off Martha's Vineyard, 1887.

Figure 2.—*Cerebratulus lacteus.* General view of a living specimen of the pink variety, natural size. Wood's Holl, July 17, 1875.

Figure 3.—*Cerebratulus luridus* V. Natural size. Noank, Conn., Aug. 8, 1874.

Figure 4.—*Cephalothrix linearis.* General view; × 8.

Figure 5.—The same. Dorsal view of the head and anterior portion of the body of a young specimen, much enlarged. Wood's Holl, Mass., August 19, 1881.

Figure 6.—*Dinophilus simplex*, sp. nov. Dorsal view, much enlarged; 6*a*, the same, ventral view of head and mouth, much enlarged. Newport, R. I.

Figures 1, 2, 3 are by J. H. Blake; 4, 5, 6 are by J. H. Emerton. All are from living specimens.

PLATE XXXVII.

Figure 1.—*Cerebratulus lacteus.* Pale variety, ½ natural size; 1*a*, the same, side view of head, in extension; 1*b*, the same, ventral view of head, in partial contraction.

Figure 2.—*Cerebratulus fuscus.* Dorsal view of head and anterior part of body in moderate extension; 2*a*, the same specimen in a state of contraction; 2*b*, 2*c*, ventral views of the same specimen in different degrees of extension. All natural size.

Figure 3.—*Cerebratulus luridus.* Natural size, but considerably contracted in length.

Figure 4.—*Lineus dubius.* Ventral view; × 2; 4*a*, dorsal view of the head, more enlarged. August 18, 1878.

Figure 5.—*Lineus viridis.* Green variety, natural size; 5*a*, the same, side view of head, natural size; 5*b*, the same, ventral view of head, more enlarged. Eastport, Me., low-water.

Figure 6.—*Micrura affinis.* Enlarged 1½; from Eastport, Me.; 6*a*, the same, posterior end of another specimen.

Figure 7.—*Micrura inornata* V. Dorsal view; × 2. Massachusetts Bay, sta. [illegible], 25 fath., 1878.

Figure 8.—*Lineus socialis.* General view of the light green variety; × 2; 8*a*, the same, side view of head and anterior part of body, more enlarged.

Figure 9.—*Lineus pallidus.* Dorsal view; × 2; 9*a*, the same, side view of head, more enlarged. Massachusetts Bay.

Figures 3, 5, 6, 9 are by J. H. Emerton; 1 and 8 by A. H. Verrill; the rest are by the author. All are from living specimens.

PLATE XXXVIII.

Figure 1.—*Nectonemertes mirabilis*, sp. nov. Dorsal view with proboscis partially extended; × 2. Atlantic Ocean, sta. 2036, 1883.

Figure 2.—*Cerebratulus Leidyi* V. Natural size; 2*a*, the same, ventral view of head. New Haven, Conn.

Figure 3.—*Micruru rubra*, sp. nov. Peculiar specimen, probably repairing mutilation of tail. Side view; × 1½; 3*a*, the same, ventral view of head.

Figure 4.—*Micrura dorsalis*, sp. nov. Dorsal view; × 1½; 4*a*, the same, ventral view of head, more enlarged. Type specimen. Eastport, Me.

Figure 5.—*Lineus arenicola* V. Dorsal view of the original specimen; × 1½; 5*a*, the same, ventral view of head.

Figure 6.—*Lineus viridis*. Red variety, dorsal view; × 1½; 6*a*, the same, side view of head; 6*b*, the same, dorsal view of a very young specimen having but four eyes; 6*c*, the same, a cluster of eggs, ⅔ natural size; 6*d*, the same, a young specimen just hatched, much enlarged.

Figure 7.—*Lineus socialis*. Young, ventral view, natural size; 7*a*, the same, dorsal view of the head and anterior part of the body, enlarged. August 12, 1880.

Figure 8.—*Lineus bicolor*. Dorsal view; × 5; 8*a*, 8*b*, the same, side and ventral views of head, more enlarged. Wood's Holl, Mass., July 14, 1875.

Figures 9, 9*a*.—*Micrura rubra*, sp. nov. Front and side views of the head; × 3. Eastport, Me.

Figures 10 and 10*a*.—*Lineus sanguineus*. Dorsal and ventral views of the head and anterior part of the body; × 3.

Figure 11.—*Lineus*, sp. (?) Young, dorsal view, much enlarged; 11*a*, another view of the front part of the same specimen. Vineyard Sound, among compound ascidians, 1881.

Figures 12, 12*a*.—*Emplectonema giganteum* V. Dorsal and ventral views of the head of the original type-specimen; × 2.

Figures 1, 6, 7, 11 are by J. H. Emerton; 8 to 8*b* are by J. H. Blake; 2 by A. H. Verrill; the rest are by the author. All are from living specimens.

PLATE XXXIX.

Figures 1, 2, 3, 4.—*Pilidium*, sp. undetermined. Different views; × 30. Wood's Holl, Mass., at surface, in day time, August 18, 1881. J. H. Emerton, from nature.

Figure 5.—The same; × 75; *a*, apical cirrus; *b*, apical plate; *b'*, nerve; *w*, head of developing nemertean with two eyes. J. H. Emerton, from nature.

Figure 6.—*Pilidium*, undetermined, sp. with golden yellow spots around the margins; taken with the preceding; *a*, cluster of apical cirri; *b*, apical plate; *b'*, nerve; *c*, *c'*, anterior and posterior lobes; *d*, *d'*, lateral lobes; *e*, œsophagus; *h*, *i*, developing nemertean; × 75. J. H. Emerton, from nature.

Figure 7.—*Amphiporus lactifloreus*. End of protruded proboscis, much enlarged; *s*, central stylet; *s'*, lateral stylets; *p*, posterior region of proboscis; *d*, bulbous region; *f*, saccular organ; 7*a*, one of the lateral stylet-sacs, more enlarged. After McIntosh.

Figure 8.—*Amphiporus ochraceus* V. Extruded proboscis, enlarged; *a*, anterior region; *s*, middle region with central and lateral stylets; *p*, posterior region. Camera-lucida drawing by the author.

Figure 9.—*Amphiporus bioculatus* (?). Middle portion of the proboscis, compressed under the microscope and much enlarged; *p*, commencement of the posterior region; *r*, muscular bulb; *s*, central stylet; *t*, one of the lateral stylet-sacs. Camera-lucida drawing by the author.

Figures 10, 11, 12.—*Cephalothrix linearis.* Different stages in the development of the larva, much enlarged; *c*, large cephalic cilia; *a*, region of the mouth; *b*, intestinal area. After McIntosh.

Figure 13.—The same, farther developed; *o*, ocelli, *h*, ganglions; *a*, mouth area; *d* opening of cephalic ducts; *m*, one of the cephalic sacs; *i*, œsophagus; *p*, proboscis; *b*, intestine, imperfectly developed. After McIntosh.

Figure 14.—*Cephalothrix linearis.* Head, much enlarged, and seen as a transparent object; *m*, mouth; *p*, proboscis; *p'*, rhynchodeum; *v*, proboscis-sheath; *b*, lateral blood vessel; *g*, superior, *g'*, inferior ganglion; *n*, origin of lateral nerve After McIntosh.

Figure 15.—The same. Part of a transverse section of the body-wall adjacent to one of the lateral nerves; *c*, external cuticle layer; *c'*, basement layer; *t*, outer, and *t'*, inner circular muscular layers; *l*, longitudinal muscular layer; *n*, lateral nerve. After Hubrecht.

Figure 16.—*Carinina grata* H. Section of a part of the body-wall corresponding to that in the preceding figure with the same lettering. After Hubrecht.

Figure 17.—*Cerebratulus medullatus* Hubr. Section of the body-wall in the region of the median dorsal line; lettering the same as in the two preceding figures, with the following additional ones; *nd*, median dorsal nerve; *n*, nervous plexus; *n'*, proboscis-nerve; *l'*, inner longitudinal muscular layer. After Hubrecht.

Figure 18.—*Lineus viridis.* Transverse section through the middle of the body. × 14; *p*, proboscis; *v*, proboscis-sheath; *b*, dorsal blood vessel; *b'*, one of the lateral blood vessels; *i*, cavity of intestine; *c*, external cuticular layer; *c'*, basement layer of cuticle; *l*, outer, and *l'*, inner longitudinal muscular layers; *t* circular muscular layer; *t'*, transverse muscular bundles arising from *t*; *n*, nervous plexus; *n'*, lateral nerve; *ro*, reproductive organs. After McIntosh.

Figure 19.—*Cerebratulus lacteus.* Transverse section of the middle region of the body; × 8. Lettering the same as in figure 18. From nature by the author.

Figure 20.—The same. Transverse section in the region of the œsophagus; × 8. Lettering the same as in figures 18 and 19, with the following additional: *i'*, plicated wall of the œsophagus; *n''*, median dorsal nerve; *u*, *u*, nephridia. From nature, by the author.

Figure 21.—The same. Portion of the same section shown in figure 20, from the region of the lateral nerve; × 36. Letters the same as in figures 18 and 20. From nature, by the author.

Figure 22.—*Lineus viridis.* Head and anterior part of body viewed as a transparent object; *o*, ocelli; *f*, *f*, lateral cephalic slits; *g*, superior ganglion; *d*, interior of olfactory sac; *d'*, its duct; *n'*, lateral nerve; *m*, mouth; *e*, œsophagus; *p*, proboscis; *p'*, rhynchodeum; *v*, proboscis-sheath; *r*, *r*, blood lacunæ surrounding the œsophagus. After McIntosh.

Figure 23.—*Malacobdella mercenariæ.* Dorsal view; × 4. Newport, R. I., July, 1880, in *Venus mercenaria.* J. H. Emerton from life.

Figure 24.—*Tetrastemma dorsale.* Central stylet; × 200; 24*a*, one of the lateral stylets; × 350. After McIntosh.

Figure 25.—*Tetrastemma candidum.* Central stylet; × 150. After McIntosh.

## ERRATUM.

Page 384, line 23, for *Nemertinea*, read *Nemertina.*

Page 405, line 31, for *candida*, read *candidum.*

## XXIII.—Dinophilidæ of New England. By A. E. Verrill.

No representatives of this group have hitherto been described from this coast, so far as I am aware. Two species have been known to me for several years, but I have delayed publishing descriptions of them, hoping to be able to obtain additional specimens in order to make the figures and descriptions more complete. But since this group is supposed by many writers to be related to the Nemerteans, it seems to me desirable that our species should be put on record in this connection.

Both our species may be referred, provisionally, to *Dinophilus*, though they differ considerably in structure. One of them (*D. simplex*) may not be a true *Dinophilus*.

### Family, Dinophilidæ Graff.

**Dinophilus pygmæus**, sp. nov.

Wood-cut 10.

Body very small, translucent, in extension long-ovate or nearly cylindrical, capable of contracting into a short-ovate or subglobular form, composed of five segments, exclusive of the head and tail; the posterior segments are usually the largest. Each segment is surrounded near its middle by a circle of rather long and strong cilia. The head is usually rounded in front, often nearly semicircular, and has a tuft or fringe of strong cilia around its front margin, and two transverse lateral tufts which are parts of two continuous preoral bands, one before and one behind the eyes. The eyes are rather wide apart, small, reniform, conspicuous.

The mouth is small and appears to be bilobed. The pharynx or œsophagus is short and swollen. On each side of the pharynx there is a small pharyngeal gland. The stomach is large, oblong-cylindrical, and occupies about three body-segments in ordinary extension; the intestine is narrow and terminates in an anal opening at the

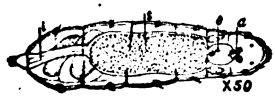


Fig. 10.—*Dinophilus pygmæus*, dorsal view, somewhat compressed; *a*, mouth; *e*, pharynx and pharyngeal glands; *s*, stomach; *i*, intestine.

base of the caudal segment, which is small, short-triangular, and terminated by a tuft of large cilia. In the posterior part of the body are two relatively large, ovate, opaque, reproductive bodies, but whether they were ovaries or spermaries I did not ascertain, so that the sex of the specimen described and figured is uncertain, but it is probably a female. Color whitish. Length ·7$^{mm}$; breadth, as compressed, ·16$^{mm}$.

Taken on the piles of a wharf at Wood's Holl, Mass., Aug. 10, 1883.

This species is closely allied to *D. gyrociliatus* of Europe. The latter has, however, six post-oral segments, and differs also in the form of the head, pharynx, and stomach. How much importance should be attached to these differences is uncertain, for they may be due largely to different conditions of the specimens examined. The two may eventually prove to be identical.

**Dinophilus simplex**, sp. nov.

PLATE XXXVI, FIGURES 6, 6*a*.

Body nearly smooth, distinctly segmented, in extension elongated and more or less cylindrical, the anterior part usually broadest, composed of four evident segments, exclusive of the large head and abortive tail. Segments well defined, but without any conspicuous bands of cilia. Head-segment large and long, subtriangular in front, and often pointed, but sometimes rounded. Eyes nearly lateral, small, but conspicuous. Mouth simple, elongated, situated between, or a little in front of the eyes. Stomach long and not much enlarged; intestine nearly as wide as the stomach, terminating in a nearly terminal anal pore. The tail segment appears to be rudimentary or abortive. The sex was not ascertained. Color pale yellow. Newport, R. I., Aug., 1880.

The affinities of this species are somewhat uncertain. The pharynx and stomach differ considerably from a typical Dinophilus. Reproductive organs were not observed.

# XXIV.—Marine Planarians of New England.

By A. E. Verrill.

## Part I.—*Dendrocœla.*

In the following paper I have brought together the scattered notes, descriptions, and sketches of our native marine planarians made by me during more than twenty seasons spent in the study of our marine invertebrates. But as the planarians were not, at any time, a subject of special investigation with me, my observations and descriptions will be found, in some instances, incomplete and unsatisfactory. At the present time other more urgent duties prevent me from making many desirable supplementary investigations of their anatomy by means of prepared sections. Nevertheless, I trust that this article, with the accompanying figures, will prove of some value as an introduction to the study of our native species of this group, which has, hitherto, been very much neglected by American naturalists.

The unsatisfactory state of what little literature there is in existence relating to our species may be, to a great extent, the cause of this neglect. I have, therefore, endeavored to unravel the synonymy, so far as it is possible to do so with the data at hand.

The drawings, except a few by myself, have all been made under my direct supervision, partly from living individuals and partly from preserved specimens rendered translucent by suitable reagents* and mounted in Canada balsam. Most of those figures (Plates 42 and 43), showing details of anatomy, and many of the general figures were drawn by my son, A. H. Verrill. Some of the general figures from life were drawn by J. H. Emerton and others by J. H. Blake for the U. S. Fish Commission.†

As a general rule alcoholic specimens of planarians cannot be identified with any degree of certainty unless rendered translucent

* In most cases the specimens have been transferred from strong alcohol to a mixture of turpentine and carbolic acid (phenol) which renders them more or less translucent; they are then mounted in balsam. Various staining agents and other reagents have also been used in making these preparations.

† For the use of the Fish Commission drawings in this place I am indebted to the liberality of the late Commissioner, Professor S. F. Baird, this article having been in preparation before his death.

by some special treatment, such as that mentioned above. When thus treated it is usually possible to identify most of the larger dendrocœlous planarians, but some of the smaller and more contractile species and most of the Rhabdocœla cannot be determined from alcoholic specimens unless studied in thin sections—a method requiring much time and giving rather uncertain results for specific identification. Such species should, therefore, be studied as fully as possible while living, and preserved by special methods on microscopic slides, so as to show their anatomy.

I have recently gone over the collections of planarians preserved in the Museum of Yale University and those contained in the collections made by the U. S. Fish Commission during many years and have identified most of the larger specimens.*

The planarians (TURBELLARIA) may be divided into three orders, viz :

I. DENDROCŒLA. In these the stomach has more or less numerous branches, often forked or arborescently divided ; the body is usually rather broad and flat. Size usually large.

II. ACŒLA. In these there is no digestive organ distinct from the body-cavity. The mouth opens into a space lined with the body-parenchyma, and without any definite bounding membrane. The body is soft, changeable, usually flat. Size small.

III. RHABDOCŒLA. In these the stomach is unbranched, and usually more or less cylindrical in form, but without any anal pore. Form of body various, often thick-ovate, and even linear. As in the preceding groups, the species are nearly all hermaphrodite. The reproductive organs are very complicated and vary greatly in structure and position in the various genera and families, so that they afford very important characters for classification. The mouth occupies various positions : anterior, medial ventral, posterior ventral, etc. Size very small. Fluviatile and marine.

The Dendrocœla are divided into two suborders, viz :

I. DIGONOPORA or POLYCLADIDEA. In these there is a central stomach from which several main branches go forth on each

* In this article all specimens recorded as collected between 1871 and 1887, unless otherwise stated, were collected by myself and others of the U. S. Fish Commission parties. The numbered stations recorded are those of the U. S. Fish Commission dredgings, made, for the most part, while I had personal charge of that part of the work of the Commission. The specimens from Eastport and the Bay of Fundy, prior to 1872, and those recorded from Long Island Sound were mostly collected by me for the museum of Yale University.

side, and also a median anterior branch; the branches subdivide arborescently and usually anastomose distally. Ovarian organs all similar; no distinct vitellaria. Male and female openings generally separate. Marine.

II. MONOGONOPORA or TRICLADIDEA. In these the central stomach is small and divides at once into three parts, viz: a median anterior, and a pair of posterior branches, from all of which simple or divided lateral branches go forth. Germaria distinct from the vitellaria. Terrestrial, fluviatile, and marine.

## DIGONOPORA Stimpson; POLYCLADIDEA Lang.

*Digonopora* Stimp., Prodromus, p. 1, in Proc. Acad. Nat. Sciences Philad., ix, p. 19, 1857.

Body generally rather large, broad, flat, often foliaceous with thin undulating margins. Very changeable in form, mouth situated on the lower side, often near the center, sometimes far forward. Pharynx sometimes long, tubular; often broad, many lobed or plicated; digestive organ has a distinct, wide central portion or stomach, from which arise more or less numerous, large lateral branches which usually fork at first, or subdivide in a dendritic manner, but in most cases anastomose distally and form a reticulated network; anterior branch of the stomach situated medially, passing above the cerebral ganglion, which is bilobed.

Ocelli usually small and numerous, variously arranged, some are generally grouped over the cerebral ganglions. Tentacles sometimes present, with or without ocelli; often absent.

Reproductive organs hermaphrodite. Ovaries and testes numerous, scattered; the ovigenous and vitelligenous organs not separate. External genital openings generally two, situated behind the mouth, the female behind the male; sometimes there are two female openings, one behind the other; rarely there are several male organs, each with a separate opening. The species are all marine.

### Tribe, ACOTYLEA Lang.

Acetabulum wanting. Pharynx short and wide; mouth in the middle of the body, or a little forward of, or behind the middle. Branches of the stomach ramose or reticulated. Copulatory organs situated in the posterior part of the body. Tentacles, when present, two dorsal, usually having ocelli; often wanting.

## Family, PLANOCERIDÆ Lang.

*Notoceridea* Diesing, Syst. Helm., p. 215, 1850.
*Planoceridæ* and *Stylochidæ* Stimp., Prodromus, pp. 4, 5, in Proc. Acad. Nat Sci. Philad., vol. ix, pp. 22, 23, 1857.

Two dorsal tentacles, usually containing ocelli. Mouth situated at or near the middle of the body. Male copulatory organ directed backward. Marginal ocelli present or absent. Cerebral ocelli generally present.

### Stylochus (Ehrenberg) Lang (restr.)

*Stylochus* (*pars*) and *Stylochopsis* Stimpson, op. cit., p. 22 [8], 1857.
*Stylochus* (*pars*) Lang, op. cit., p. 447.

Body very changeable, usually ovate or elliptical and flat in full extension, but thicker and firmer than usual in this group when contracted. Two dorsal tentacles, each containing a conspicuous group of ocelli, and situated rather far back from the anterior end of the body. Other groups of ocelli are situated between, or in front of the tentacles over the cerebral ganglions and frontal nerves; others form submarginal rows, most numerous anteriorly. The pharnyx is furnished with several accessory plicated lobes or pouches. Male and female genital openings are situated close together near the posterior end of the body and in the typical species open into a common external pit or pore; female copulatory pouch apparently wanting in the typical species; accessory vesicle or spermatheca absent or small.

The following species are referred with some hesitation to this genus, with which they agree well in external characters. They differ from the figures and descriptions given by Lang of the European species in the structure of both male and female copulatory organs, especially in having two distinct genital openings, with the female duct opening backward. I have, accordingly, modified the generic characters so as to include them. But when their anatomy becomes better known it may be found that they belong rather with the new genus *Eustylochus*, described below. At present it seems best to put them in the older genus, which still evidently includes very diverse species.

One of these peculiar forms I have separated (see p. 466, note) as a new genus, *Heterostylochus*.

The anatomy of both the type-species of *Stylochopsis* Stimp., is still unknown. There is nothing in the original diagnosis of that group to distinguish it from *Stylochus*, as now restricted.

**Stylochus zebra** Verrill.

*Stylochopsis zebra* Verrill, Amer. Journ. Sci., vol. xxiv, p. 371, 1882; Annual Report U. S. Com. of Fish and Fisheries, for 1882, p. 666, 1884.

PLATE XL, FIGURE 3; PLATE XLII, FIGURES 2, 2*a*.

Size large. Body thick and firm, when active oblong or long-elliptical, usually rounded at both ends; in full extension often five or six times as long as broad, but capable of contracting to a broad oval or elliptical form. In full expansion the body is rather thin, though convex dorsally, but is nearly opaque, except at the margins, which are translucent and often more or less undulated. In strong contraction the body is convex, thick, and firm. Tentacles situated not far from the anterior end, at about the anterior eighth or tenth, and not far apart; they are short, obtuse, white, filled with very numerous, crowded, minute, black ocelli. Two large, rather open, elongated cerebral clusters, composed of numerous very minute ocelli, are situated between the tentacles and extend about the same distance forward and back of them; the hind ends of these clusters are widest and contain the greatest number of crowded ocelli; the two clusters often blend, more or less, on the median line, and terminate posteriorly rather abruptly. In some specimens these clusters are imperfectly developed, with comparatively few ocelli; in others, of large size, additional scattered ocelli occur over the whole anterior end, between the cerebral groups and the marginal rows. The marginal ocelli are minute and very numerous; around the anterior end they form three or four irregular rows, but they extend in one or two rows to the posterior end. The pharyx, as contracted, is elongated and has five or six pairs of much divided lobes anteriorly, and three or four pairs of successively smaller and simpler lobes along the sides of the elongated posterior portion, which ends not far from the middle of the body. Mouth anteriorly situated about opposite the third pair of large stomach-branches and pharyngeal lobes. There are, evidently, two genital pores, only a short distance apart and inconspicuous.* The *vas deferens* extends forward to about the posterior third of the stomach as a conspicuous

* I have already stated, on a former page, that this is not a typical species of *Stylochus*, as that genus is defined by Lang, for it differs considerably from all the European species in the structure of the copulatory organs, and especially in having two separate genital openings. But our other species seem to agree with this in that respect. It might be regarded, quite as well, as a *Planocera with marginal ocelli* and numerous tentacular ocelli.

convoluted, opaque white organ, on each side. Shell glands show through faintly as a cloudy cluster of grayish white, slender, branched, radiating tubes. The other reproductive organs show only obscurely in adult living specimens, owing to the opacity and thickness of the integument. The interior throughout most of the body is filled with great numbers of ovarian eggs.

Color, above, yellowish brown, umber-brown, or chocolate-brown, crossed by numerous yellowish or whitish lines and stripes, of which there are sometimes at least fifty, while many other similar but narrower and shorter stripes run in from the margins, between the main ones, usually narrowing and disappearing before reaching the middle of the body; toward the ends of the body the light stripes diverge more and more till those of opposite sides form v-shaped markings at each end, with a small median white stripe at the extreme end. Sometimes the light stripes become wider as they approach the middle of the back, as in the specimen figured on Plate XL, fig. 3. Some examples show an ill-defined median pale stripe posteriorly. The under side is mostly pale buff, except close to the margins, which show the alternation of brown and white stripes by translucency; the pharynx, stomach lobes, and *vas deferens* are opaque white in life, and show distinctly on the ventral side. Described from adult living examples.

Length, in life, 30 to 40$^{mm}$; breadth 10 to 12$^{mm}$ or more.

Wood's Holl, Mass., on piles, and on the shore of Great Harbor at low-water, 1882; Vineyard Sound, off Menemsha, in 10 to 12 fathoms, September 6, 1886. Several fine specimens were taken on oyster beds in Long Island Sound, off New Haven, by Mr. Gilbert Van Ingen, October 29, 1892. These were all found in the canals of dead shells of *Fulgur* that were occupied by hermit crabs (*Eupagurus pollicaris*). Some of those previously taken in Vineyard Sound were living in the same situation.

Owing to the density of the pigment and the opacity of the tissues, I have not been able to make out satisfactorily the structure of the reproduction organs in this species, without sections, for which I have not yet had sufficient material in good preservation. There are, apparently, two small genital pores situated a short distance apart and relatively farther forward than in our other species of this family.

This species can always be recognized at once by its peculiar and conspicuous colors. It is, also, the largest planarian commonly found on our coast. The color is usually recognizable, even in alcoholic specimens, as well as in those mounted in balsam as translucent

objects. From our other species of the family it also differs strongly in the more anterior position of the mouth and stomach, as well as in the arrangement of the frontal and cerebral ocelli.

**Stylochus frontalis** Verrill, sp. nov.

PLATE XLIV, FIGURE 1.

Body elliptical, large, about one inch long and half as broad. Tentacles small, obtuse, near together, well forward, nearly surrounded at the base with a ring of many ocelli, and containing very numerous, small black ocelli, arranged in two groups, one on the antero-lateral, and the other on the inner-lateral surface. Frontal, and cerebral ocelli form a large, median, double cluster containing very many small ocelli, situated before and between the bases of the tentacles and running to a point back of them, so as to form a median triangular patch, and extending forward in the form of a fan, or large open triangular group, formed by the nearly complete blending of the two lateral clusters, and becoming more widely scattered toward the anterior margin. Marginal ocelli minute, forming two or more closely crowded rows, close to the edge and most numerous on the middle part of the anterior border, but extending to the sides.

Color, above, yellowish gray, mottled with yellowish brown, and marked with the brighter orange-colored, repeatedly forked and branched intestinal branches, which show best toward the paler margins, and with unequal, rounded, scattered spots of dark brown around the central region. The pharynx and stomach cause a central whitish patch, narrowing backward; behind this there is a small pale patch, near the posterior end, caused by the reproductive organs. The under side of the body is paler than the upper.

Length, in life, about $25^{mm}$; breadth $12^{mm}$. Described from life.

One living specimen was taken at Provincetown, Mass., from the bottom of a whaling vessel recently returned from a cruise off the Carolina coast in 1879. Some of the associated species were of distinctly southern origin.

The reproductive organs were not well seen, owing to the dark color of the pigment. There appear to be two genital openings, near together. The mouth is in advance of the middle of the body. The pharynx has about five principal branches on each side, decreasing backwards, while the posterior end extends some distance beyond the last branches. The branches of the stomach are much divided dichotomously, but do not seem to anastomose to any great extent.

**Stylochus crassus** Verrill, sp. nov.

*Description of a specimen preserved and rendered partially translucent:*—Body large, thick, rounded, nearly as broad as long. Upper surface covered with minute, whitish, conical papillæ, barely visible to the naked eye. Mouth nearly central. Stomach with about five or six main lateral branches, which have very numerous divisions and anastomose freely distally. A short distance behind the mouth there is a rather large and slightly prominent sucker-like organ or acetabulum.

Ocelli are minute and very numerous, forming two large, elongated, sub-parallel groups, broadest posteriorly and tapering anteriorly; at its posterior end each group expands into a wide, roundish, rather open cerebral cluster, which is connected by a narrow band of ocelli with another similar roundish cluster a little farther forward, over the anterior part of the brain, beyond which the groups gradually diminish in breadth to their anterior ends, not far from the front margin of the body. At about the anterior fourth, and a little back of the posterior part of the cerebral groups, and somewhat farther apart, are two low, inconspicuous prominences, which appear to be the retracted tentacles; each of these contains a small group of minute ocelli, not easily distinguishable.

Marginal ocelli are minute and not very numerous, forming about two rather irregular and indistinct rows anteriorly, but the margin is somewhat abraded and some of its ocelli may have been destroyed.

Most of the interior of the body is densely filled with small ova. The density, thickness, and opacity of the tissues render the study of the reproductive organs impossible, except by sections, for which my single specimen is not suitably preserved.

Color of the preserved specimen dull brown, covered above with small white specks, due to the papillæ.

Length and breadth of the alcoholic specimen about $25^{mm}$; thickness 2 to $3^{mm}$.

Station 2732, U. S. Fish Com. steamer Albatross, Oct. 26, 1886, N. lat. 37° 27′; W. long. 73° 33′, in 1152 fathoms, dark green mud.

Only one specimen was taken. It is remarkable for its great size, thickness, and solidity. I refer it to this genus only provisionally, because its reproductive organs are unknown. I detected only a single genital pore, of small size, near the posterior end, but a second may exist, for the specimen is broken and not well preserved in that region. The arrangement of the ocelli is sufficient to distinguish it from all other American species.

It is possible that it lives at the surface among floating algæ, and not at the bottom.

**Eustylochus**, gen. nov.

Tentacles with ocelli in the sides, or base, or both. Cerebral ocelli variously arranged. Marginal ocelli present. Stomach branches numerous; distal branches anastomosed.

Male and female genital pores separate, the female ducts opening backward. Seminal vesicle well developed, usually distinctly three-lobed, the middle lobe with a long duct leading to the penis. Penis-sheath short and thick; penis styliform; granular gland not prominent, closely attached to the penis-bulb, often indistinct.

Female orifice not far back of the male organ; it connects with the vagina and with a long, narrow median duct which starts from the orifice and runs forward to or near the gastric region, where it connects with a flask-shaped vesicle, which is probably a spermatheca or seminal receptacle. The vaginal duct expands into a short, swollen glandular portion, which bends upward and backward upon itself, and receives the uterine ducts.

This genus agrees externally with *Stylochus*, but its reproductive organs are very different from those of that genus.

The only European species which is described as having a similar spermatheca and median duct is *Stylochus maculatus* Quatrefages,* but that differs in other characters.

**Eustylochus ellipticus** (Girard) Verrill.

*Planocera elliptica* Girard, Proc. Boston Soc. Nat. Hist., vol. iii, p. 251, 1850 (description very brief and imperfect); op. cit., p. 348 (note on its embryology). Embryonic development of *Planocera elliptica* in Journal Acad. Nat. Science of Philadelphia, vol. ii, p. 307, sep. copies, pp. 7-27, pl. 1-3, 1854 (details of its embryology, but no description of adult).

Stimpson, Prodromus, in Proc Philad. Acad. Nat. Sci., vol. ix, p. 25 [5], 1857.

Lang, Polycladen des Golfes von Neapel, p. 463, 1884 (copy of Girard's 1850 brief description).

*Stylochopsis littoralis* Verrill, Report on Mar. Invert. of Vineyard Sd., etc., pp. 325 [31], 632 [338], pl. 19, fig. 99, 1873.

*Stylochus littoralis* Lang, Polycladen des Golfes von Neapel, p. 453, 1884 (copy of description in work last cited).

PLATE XL, FIGURE 2; PLATE XLI, FIGURES 1, 1*a*; PLATE XLII, FIGURES 1, 1*a*.

Body flat with thin, often undulated, and very flexible margins; very changeable in form, usually, when creeping, long-oval, elliptical, or oblong; in extension often narrowed anteriorly, but when at rest rounded or subtruncate at the ends; often with a small median

* This species, as described and figured by Quatrefages, differs so much from all related forms, that it should, undoubtedly, constitute the type of a new genus, for which I propose the name *Heterostylochus*. Some of its characters are as follows:

posterior lobe, but at other times with a notch in the middle of the posterior margin; frequently the breadth exceeds the length, but more commonly it is less than half. The tentacles are small, round, elongated and tapered in full expansion, often obtuse in partial retraction, translucent white, each containing an elongated, crowded group, extending from the base nearly to the tips, consisting of about twelve to twenty-five small, but conspicuous, black ocelli. The tentacles are situated at about the anterior fourth of the body, and are usually separated by a distance equal to about one-fourth of its breadth, but the relative positions of the tentacles change according to the degree of extension. Frontal and cerebral ocelli usually about eight to twelve, often more in the largest specimens. They usually form four or six small paired groups; the two groups most in advance of the tentacles and situated over the frontal nerves sometimes contain three or four ocelli each, but more commonly only two; the next pair of groups, which are nearer together and situated between or a little in front of the tentacles, about over the apex of the cerebral ganglions, or near the base of the large frontal nerves, usually contain each only two or three ocelli, one of which is larger than the others, but in some adult specimens they contain four or five ocelli, the number in that case often varying in the right and left clusters; a little farther back and directly over each gangloin there are often one or two pairs of ocelli; occasionally there are others placed singly over the posterior part of the gangloins. Young examples often have only the two front pairs of groups, each consisting of two ocelli. Marginal ocelli numerous, black, easily seen with a lens; they are most numerous on the anterior margin, where they are arranged mostly in two or three or more irregular rows along the pale border; they extend back to the middle of the sides or beyond, gradually decreasing in numbers backward; a few ocelli, which are usually loosely arranged somewhat in radial rows over the

*Heterotsylochus*, gen. nov.

Tentacles with lateral ocelli; cerebral ocelli form two groups; apparently no marginal ocelli. Main stomach-branches few. Genital openings separate. *Vasa deferentia* large, discharging into the ejaculatory duct; seminal vesicle large, rounded, sessile. Muscular penis-bulb pyriform. A long, narrow, median duct (*vagina* Quatr.), runs far forward from the female orifice and expands into a flask-shaped seminal receptacle or spermatheca near the male organs; a swollen egg-duct also connects with the female orifice.

Type *H. maculatus* (Quatr. sp.) from Saint Malo, under stones. (See Ann. des sci. nat., iv, p. 144, pl. IV, figs. 3, 3*a*; pl. VI, fig. 2, anatomy).

*Stylochoplana maculata* Lang, op. cit., p. 459.

frontal nerves, anteriorly, but often appear irregularly scattered, occur farther from the margin than the main rows.

Mouth submedian, or at about the anterior third. Pharynx, when everted, short and broad, with numerous short, flattened lobules. The retracted pharynx has about six or seven pairs of principal lateral lobes and some smaller ones anteriorly and posteriorly. The six or seven pairs of lateral branches of the stomach, and also the anterior and posterior ones, have many dendritic branches at first, but distally anastomose freely making an intricate network.

The copulatory organs lie in an elongated whitish spot, close to the posterior end. The most anterior part of this spot contains an elongated, swollen, median seminal vesicle, which tapers backward, and has two lateral lobes, continuous with the *vasa deferentia;* back of this the small, opaque white, elliptical or barrel-shaped male organ is more or less distinctly visible ; the penis is short, straight, tapered, and appears to end at a small distinct external pore. The *vas deferens* extends forward, on each side, to the middle of, or sometimes nearly to the anterior end of the stomach, in the form of a much convoluted opaque white organ, often distinctly visible from beneath, through the integument, even with the naked eye.

Just back of the penis, and more indistinct, there is a smaller, swollen, short, pear-shaped female copulatory organ (vagina or copulatory pouch) which has thick, rather opaque, glandular walls ; it is surrounded by, and connected with a larger translucent organ or cavity, and opens externally by the small genital pore at its posterior apex. The glandular part of the vagina, in most preserved examples, appears to be short, ovate or pear-shaped when seen from the ventral side, but its front end bends upward and then backward on itself, the dorsal bend being of nearly the same shape and size as the ventral part, is mostly concealed by it ; the apex of the dorsal fold, in the largest examples, again bends forward, so that the entire organ in profile, is somewhat S-shaped, but this is not the case in some of the smaller specimens. From the external female orifice a narrow but very definite duct runs forward, in the median line beyond the penis and seminal vesicle to the gastric region, where it expands into an elongated flask-shaped vesicle, which lies below the stomach.* This appears to be a seminal receptacle or spermatheca.

* An organ precisely like this is figured by Quatrefages (op. cit., pl. VI, fig. 2*l*), as present in his *Stylochus maculatus* (*Heterostylochus maculatus* V.), as described in note on page 466. By him it was considered a copulatory sac or seminal receptacle, and he called its duct the vagina. Others have doubted whether it forms part of the

Close to the female genital orifice there is a minute pore, from which runs forward a long narrow duct (often distinctly stained with carmine in the preparations). This appears to be the main nephridial duct; it was traced as far as the gastric region.

Color pale or deep yellowish brown, greenish brown, salmon, smoky brown, greenish yellow, etc., irregularly radially veined or reticulated with a pale, translucent, yellowish, whitish, or salmon ground-color and usually with a light brown, yellow, or whitish, irregular, laterally lobed median stripe, most distinct posteriorly; a small posterior pale spot; margins pale. In some examples the yellowish brown color appears in the form of thickly arranged, irregular, angular spots and patches on a pale translucent ground, while the stomach, pharynx, and reproductive organs (*vas deferens*) cause an elongated, irregular, lobulated, whitish median patch, more distinct on the under side; beneath white, pale gray, or pale flesh-color, or salmon, with a median, elongated, lobulated, light patch, due to the stomach and *vasa deferentia.*

Length of adults 20 to $25^{mm}$ in extension; breadth, about 6 to $10^{mm}$.

New Haven, Conn., to Casco Bay, Maine, common in shallow water and between tides, under stones, and in tide-pools.

Fort Hale, South End, and Savin Rock, near New Haven; Thimble Islands, abundant; Newport, R. I., on piles of bridges and wharves, 1880; Wood's Holl and Vineyard Sd., Mass., common; Provinctown, Mass., at low-water mark, common, Aug., 1879; Salem and Gloucester, Mass., on piles, 1877, 1878. Quahog Bay, Maine, at low-water, 1873; Banks of Newfoundland (?), T. M. Coffin. A number of large specimens were taken by Mr. Gilbert Van Ingen, Oct. 29, on the piles of the railroad bridge across West River, near New Haven, where the water is distinctly brackish. Each one was inside the shell of a recently killed barnacle (*Balanus eburneus*) and was evidently feeding upon its flesh. The barnacles may, however, have been killed by some other agency.

A specimen taken at Savin Rock, near New Haven, laid its eggs on July 12th in large clusters placed close together on the inside of a glass bottle. The eggs themselves were very small and white.

This is decidedly the most common species of the larger planarians found on the shores of New England, especially south of Massachusetts Bay. I have not obtained it during many seasons of collecting

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reproductive system. In our species its connection with the orifice that I take to be the female genital pore is very obvious in some prepared specimens, in which the duct has taken a deeper stain than the surrounding parts.

at Eastport, Me., nor elsewhere in the Bay of Fundy. Therefore the specimen received from Capt. Coffin marked as from the Newfoundland Banks may be regarded as doubtful, as to locality.

I have referred this species to the *Planocera elliptica* of Girard with much hesitation, for Dr. Girard did not publish any description of his species by which it could be identified. He did not even mention the arrangement of the ocelli in either of his papers, but said, incidentally, that he understood the genus *Planocera* as including *Stylochus*. Hence it may be inferred that his species had ocelli in the tentacles, but not necessarily marginal ocelli. My principal reason for uniting *S. littoralis* with his species is because it has proved, in my experience, to be the common shore species in the region of Massachusetts Bay, where Girard obtained his specimens. Nor have I found in that district any other species which could be referred to his species with any degree of probability whatever. As Dr. Girard published an important paper on the embryology of his species, it is very desirable to retain his specific name, if it can be located with any considerable degree of certainty.

**Planocera** Blainv., 1828 (emend.)

*Planocera* Stimpson, Prodromus, p. 5, 1857 (emend.)
Lang, Polycladen, p. 434, 1884 (emend.)

Body oval or elliptical and rather flat in extension, thickened and convex when contracted. Tentacles slender, tapered, retractile, situated at some distance from the anterior end of the body. A cluster of ocelli is generally situated in the base of each tentacle; usually others form clusters between and behind the tentacles; marginal ocelli wanting in the typical species, sometimes numerous. Genital pores separate, but near together, situated a short distance from the posterior end of the body; female duct is short U-shaped, usually with a copulatory pouch and accessory vesicle (spermatheca); the vagina opens backward; the spermatheca is above or behind the vagina.

I have modified the generic characters so as to include species which, like the following, have marginal ocelli, but otherwise agree nearly with the typical species. For this group I propose, however, to establish a new subgenus:

Subgenus, **Planoceropsis**, nov.

Tentacles, cerebral ocelli, pharynx, and stomach as in typical *Planocera*; marginal ocelli present. Reproductive organs mostly

similar to those of typical *Planocera*, but the seminal vesicle is free and three-lobed, with the central lobe elongated, tapering backward into a narrow duct that joins the penis, while the side lobes are continuous with the *vasa deferentia;* the penis-sheath is short, thick, conical; the penis is short, styliform. Female duct runs forward a short distance from the opening and then bends upward and turns back on itself, forming a U-shaped tube.

### Planocera nebulosa Girard.

*Planocera nebulosa* Girard, Proceedings Philadelphia Academy Natural Sciences, for 1853, vol. vi, p. 367, 1854; Verrill, Report on Invert. of Vineyard Sd., etc., in Annual Report U. S. Com. of Fish and Fisheries for 1871, pp. 325 [31], and 632 [338], pl XIX, fig. 100, 1873; Lang, Polycladen, p. 463.

PLATE XL, FIGURE 4; PLATE XLII, FIGURE 3.

Body convex above and rather thick, especially in contraction. In usual extension, broad-elliptical or oblong, but capable of becoming long-elliptical or nearly circular; edges sometimes thin and undulated. Tentacles rather long, slender, tapered, translucent whitish, retractile, situated near together and well back from the front margin; no ocelli in the distal portion, but a cluster of small ocelli is situated in the base. Mouth and stomach nearly central; the pharynx and stomach have four or five main lobes on each side, and the mouth is a little behind the middle of the stomach; in some states of expansion the anterior and posterior lobes are somewhat prolonged. The bilobed cerebral ganglion is situated between or a little before the bases of the tentacles. No ocelli were observed in the deep colored living specimens, above described, but when looked for in sections they are found to be numerous. The ocelli in the bases of the tentacles form small rounded clusters, more easily visible from below than from above. Just back of each tentacle and a little more toward the median line there is a larger and more open cluster of ocelli; in the cerebral region there is a pair of small inconspicuous groups, each containing about two or three ocelli, while a few others are apparently irregularly scattered between and in front of the tentacles. The marginal ocelli are also minute and form two or three rows, some of which extend back to about the middle of the sides.

Genital orifices two, small, near together, not far from the posterior margin.

Color somewhat variable. Our specimens were usually olive-green with the paler ground-color showing more or less as whitish or

yellowish lines and mottlings; median dorsal stripe pale or greenish white; margin whitish; sometimes the dark color is in the form of distinct irregular spots or blotches; ventral surface yellowish green, with the pharynx opaque whitish.

According to Girard the original specimens were dark gray, on a pale ground, and the median stripe was reddish,—a variation similar to that common in *Eustylochus ellipticus.*

Length $20^{mm}$; breadth 10 to $12^{mm}$, in extension.

New Haven, Conn., 1865, 1870, 1892; Newport, R. I., 1880; Wood's Holl., Mass., under stones near low-water mark, 1886; Charleston, S. C. (Girard.)

On the southern New England coast this species is not common,—at least I have found it only in few instances and in small numbers during more than twenty seasons spent in collecting marine animals on these shores. Most of the specimens are immature.

The structure of the internal reproductive organs of this species, owing to its opacity and dark color, were not well made out, in the adult specimen described above. Some additional observations on a smaller and somewhat immature specimen, apparently of the same species, recently obtained living, are here given.

This specimen agrees with the larger and more characteristic ones described above in the arrangement of the ocelli, which, owing to the lighter colors of the integument, could be made out in the living specimen, though they are minute. The tentacles, however, were relatively smaller than in the larger specimen formerly described. They are tapered, acute, translucent whitish, without ocelli except in the strictly basal part, so that they can scarcely be seen from above, but seen from beneath they form a small round group; the clusters behind and between the tentacles, those in the cerebral region, and the marginal ocelli are nearly as described above, except that they are rather less numerous. They can hardly be seen with a pocket lens. Scattered, minute, marginal ocelli occur even at the posterior margin. The color is light olive-green, irregularly radiated and reticulated with rather faint, greenish white lines; margins and tentacles whitish; a median streak over the stomach and a small elliptical spot close to the posterior end, and inclosing the copulatory organs, are greenish white. Lower surface pale, the pharynx, stomach, and *vas deferens* showing through as more opaque white organs. Mouth central; pharynx with about six main lobes on each side.

In this living specimen the reproductive organs (Pl. XLII, fig. 3)

could be seen indistinctly when it was compressed under the microscope. There are apparently two small genital pores, near together, but distinct, as in *E. ellipticus*, with which this species seems to agree nearly in the structure of its reproductive organs. The convoluted *vas deferens*, which runs up, on each side, to about opposite the middle of the stomach, discharges into the lateral lobes of a relatively large, three-lobed, or somewhat anchor-shaped, seminal vesicle, which is more conspicuous than most of the other organs; its three lobes point backward, while its anterior end is broadly rounded; its middle lobe is longer than the others; and connects with a narrow efferent duct. The male copulatory organ is broad-elliptical, terminating in a conical penis-sheath and a small, short, straight, tapered penis, which terminates close to a small external pore. The larger or elliptical part of the male organ may be, at least in part, a "granular gland," but I was unable to clearly distinguish between the penis-bulb and the glandular organ. The female organs consist of two evident parts; the more anterior portion is a small elliptical vesicle (copulatory pouch ?); with a posterior pore; the posterior portion is a narrower and more tubular organ, which appears to be a simple spermatheca. This specimen is not mature sexually and the female organs are not well developed.

I have referred this, with considerable doubt, to Girard's species, for the description of the latter was very meagre, and there is considerable difference in color. The color, however, is quite variable in this group. The southern form may prove to be a very distinct species.

The generic relations of this species are somewhat doubtful. In general structure, and especially in the characters of its tentacles and dorsal ocelli, it agrees well with the typical species of *Planocera*, but the latter genus is described by Lang and others as destitute of marginal ocelli, which are numerous in our species. In the structure of the reproductive organs it agrees more nearly with *Planocera* than with *Stylochus*, as described by Lang.

In many respects the reproductive organs are like those of *Eustylochus*, and it is possible that its affinities may be even greater with that genus than with *Planocera*. But as there is but a single specimen available for the study of the reproductive organs, and that seems to be immature, I am unable to determine some of the details which are desirable before its position can be fully established.

The presence or absence of marginal ocelli might be regarded as not of generic importance. In that case the present species might be defined as a *Planocera* with marginal ocelli.

**Imogine** Girard, 1853.

*Imogine* Stimpson, Prodromus, p. [4] 22, 1857; Lang, Polycladen, p. 445.

Body thin, foliaceous. Tentacles with a single rather large ocellus at the tip. Anterior border furnished with marginal ocelli. Reproductive organs unknown.

**Imogine oculifera** Girard.

*Imogine oculifera* Girard, Proc. Philad. Acad. Nat. Sci., p. 367, 1853; Stimpson, Prodromus, p. [4] 22; Lang, op. cit., p. 446.

PLATE XL, FIGURE 1.

The only specimen that I have seen was small and immature. The reproductive organs were not developed. The body of this was very thin, obovate, with a broadly rounded anterior and a narrowed posterior end, when creeping. The tentacles were distant from the front margin, clavate, each with a very distinct ocellus in the rounded tip. The ocelli formed two linear divergent groups of about ten each, commencing between or a little behind the tentacles and extending much beyond them anteriorly. The pharynx and stomach were not well defined, but the stomach appeared to have rather numerous (at least seven or eight) main lateral branches, which were much divided distally, and appeared to branch dichotomously.

The color, to the naked eye, appears bright red with pale margins; when much enlarged the bright carmine red is seen to form irregular radiating and branched lines, corresponding to the branches of the stomach, while the narrow intervening spaces, the gastric region, the cerebral area, the tentacles, and the margins are translucent whitish. The margin is, however, covered with small pale yellow spots.

Length of the young specimen described and figured, in life, $4{\cdot}5^{mm}$; greatest breadth $1{\cdot}5^{mm}$. According to Girard, this species becomes 1·5 inches long; ·5 wide.

Buzzard's Bay, at Quisset Harbor, in 4 or 5 fathoms, on sandy bottom, Sept. 4, 1882. Charleston, S. C. (Girard).

Family, LEPTOPLANIDÆ Stimp., 1857.

Body broad, flat, usually thin or foliaceous. Tentacles none. Mouth ventral, near the center of the body. Pharynx large, lobed or plicated. The main lateral branches of the stomach vary in number; the distal branches often anastomose freely. Ocelli nu-

merous, usually forming four groups, two of which are cerebral, and two dorsal in the position of the tentacles of *Planocerideæ*. Marginal ocelli sometimes present, often wanting or indistinct. The copulatory organs are situated behind the mouth and more or less distant from the posterior end of the body. The male organ is directed backward. Male and female genital pores are usually considerably separated, but sometimes have a common external opening. In some genera a second female orifice is situated behind the ordinary one. No marked metamorphosis.

The species and genera of this family are not easy to distinguish without a careful study, both of the living and prepared specimens. The majority of the species, while living, closely resemble each other in form, color, habits, etc., although the internal organs may show great diversities in arrangement and structure. Unfortunately it is generally difficult, if not impossible, to make out much of the internal anatomy while the animals are alive, owing to the thickness, density, and imperfect translucency of the tissues; the presence of large quantities of ova; the frequently deep colors, etc. On this account the species, as collected, are frequently confounded, and the rarer species, being mistaken for common ones, are often overlooked. The only sure way, therefore, is to save all the specimens seen.

Even the arrangement of the ocelli is apt to be deceptive, for many very diverse species have the ocelli arranged in almost exactly the same way. Moreover the number of ocelli and the forms of the clusters in each species vary widely, according to the age of the individuals; and the form and relative positions of the clusters of ocelli change greatly according to the states of contraction and expansion. In life, part of the ocelli are apt to be overlooked on account of their being more or less deeply imbedded in the integument, and perhaps, also, partly concealed by pigment, as well as because of their very small size in some species. The marginal ocelli, especially, are liable to be overlooked in several species, owing to their minuteness. I have found it impossible to see the marginal ocelli with the best of simple lenses, in some of our species, while they were alive, but could see them easily when the same specimens were properly preserved and mounted.

The form is continually changing in life, and preserved specimens are apt to contract in all sorts of shapes, unless care be taken to confine them between glasses before killing.

The colors of each species, in life, are usually variable through a wide range of tints, and the special color-markings, when due to the

branches of the stomach, or to the reproductive organs, vary according to the kind and quantity of food, the season of the year, etc. Hence the forms and colors are usually of secondary importance in distinguishing the species and genera.

The form and structure of the reproductive organs are here of the greatest importance, but these organs can usually be seen only very imperfectly, if at all, unless the specimens are preserved and mounted in such a manner as to render them translucent.* A few species are sufficiently translucent while living, especially when immature, to afford a fairly good view of their internal organs when slightly compressed between glass, especially immediately after they are killed with some agent that does not coagulate the fluids of their bodies.† The structure of the pharynx and the number and modes of division of the main branches of the stomach afford characters of great value that can be easily observed.

I have good reasons for beliving that there are a number of species of this group living on our coast, that are not included in this article. This is doubtless largely due to lack of attention to this family on the part of collectors, myself included, owing partly to the prevalent impression that all the forms are members of one or two common species. I have observed young specimens of several kinds, too immature to describe specifically, that are evidently not the young of any of those here included.

**Leptoplana** (Ehrenberg) Lang, (restr.).

*Polycelis* (*pars*) Quatrf., Ann. des sci. nat., ser. 3, vol. iv, p. 133, 1845; Voyage en Sicile, ii, p. 33, 35.

*Leptoplana* and *Elasmodes* (*pars*) Stimp., Prodromus, p. 3, 1857.

Body foliaceous, usually with thin undulated margins, usually elliptical or oblong, changeable. Ocelli numerous, forming four groups : cerebral and dorsal. The anterior or cerebral ones are situated over the cerebral ganglions, the dorsal groups occupy the position of the tentacles found in *Planoceridæ*. No marginal ocelli. Pharynx with more or less numerous broad, short accessory lobes.

Genital pores rather widely separated, the male pore distant from the posterior end of the body. Female pore opens backward and is

* It must be noted that the form and relative positions of these organs are more or less altered by all modes of preservation, owing to contraction of the tissues.

† I have found hydrogen peroxid an excellent reagent for this purpose. Nitric acid often does very well, as do many other agents in common use. Alcohol, corrosive sublimate, chromic acid, etc., render the tissues more opaque.

often in a circular pit; the female duct is usually elongated and forms a siphon-shaped organ, the longer and narrower leg of the siphon above the vagina, and extending backward into a more or less dilated spermatheca; the middle portion of the vagina* is thickened and receives, on each side, numerous ducts from the shell-glands. In at least two of our species there appears to be a small posterior genital pore connected with the spermatheca by a narrow duct, somewhat as in *Trigonoporus.*

A large, round or pyriform, thick-walled "granular gland" is connected with the male organ. Seminal vesicle usually well developed. Penis or verge often long and cirriform. In some species a sucker exists between the genital pores.

The most obvious distinction between this and the closely allied genera is the unusual elongation of the female ducts and the verge, and the consequent wide separation of the genital pores. The absence of marginal ocelli seems to be constant.

## Leptoplana virilis, sp. nov.

PLATE XLIII, FIGURES 1, 1*a*.

*Description of living specimens:*—Body thin, more or less elliptical, changeable. Ocelli conspicuous, black, arranged in four distinct, rather large groups, those in the posterior or dorsal groups largest; the posterior groups are short-oblong or somewhat quadrangular, divergent, situated near the ganglions, and have each about twelve visible ocelli; the anterior or cerebral groups are nearly parallel, elliptical or oblong, a little nearer together than the others, and usually commence between the latter and extend considerably farther forward over the ganglions; they consist of many minute ocelli and each has a single distinct ocellus, larger than the others, at the posterior, and another at the anterior end of the group.

Color pale brown covered with darker brown specks and with poorly defined pale blotches.

Length $18^{mm}$; breadth $10^{mm}$ in extension.

*Description of a specimen mounted in balsam:*—This specimen (pl. XLIII, figs. 1, 1*a*) shows that the ocelli are somewhat more numerous than described above, part of them being too deeply seated to

* The first, or thickened, glandular portion of the duct (*v*, pl. LXIII, fig. 1*a*) is usually called the *vagina*, but it may not be the copulatory duct, at least in such species as have a second female orifice farther back leading to the spermatheca, as in *Trigonoporus.* The latter duct, in such cases, is probably the true copulatory duct, the other serving for the laying of the eggs.

be readily seen in life; the anterior clusters are shorter than in life, owing to contraction. The retracted pharynx has five pairs of large lateral lobes, and a pair of smaller ones anteriorly. The mouth is about opposite the second pair of large lobes.

The greater part of the body is filled with small rounded ovarian folicles (*o*, *o*) crowded between and around the branches of the stomach. A still larger number of spermaries (*t*, *t*), of smaller size, are arranged around and between the ovaries.* Only a part of them are represented in the figure.

The male organs of copulation are larger than in most species and rather conspicuous. The muscular penis-sheath (*q*) is stout-cylindrical, or slightly clavate, longer than broad. Its anterior part is covered by a nearly globular "granular gland" (*k*) with thick, dark colored walls. The seminal vesicle (*r*) in a ventral view appears like a rounded cap anterior to, and above the granular gland; it consists of a median and two lateral lobes. The penis (*p*) is long, slender, and somewhat enlarged at the end, as seen in this example, in which it is retracted and probably unnaturally crooked and twisted, owing to the mode of preservation.

The female genital pore is situated at the bottom of a large and deep funnel-shaped pit (perhaps produced by contraction), and is situated about a third of the distance from the male pore to the end of the body. The female duct (*v*, *v'*) extends forward to near the male pore where it bends upward and turns abruptly backward on itself, and then becoming a narrow tube (*v'*), runs backward considerably beyond the male genital pore and connects with a rather large and nearly round spermatheca or *receptaculum seminalis.*

This organ (*s*, *s'*) seems to consist of two parts or compartments, for in some specimens there can be seen a smaller rounded organ (*s'*) overlapping or resting upon its anterior side and apparently having its cavity continuous with the other. In other specimens it is in front of the main vesicle and seems joined to it by a neck. These parts are, however, much obscured by the glandular organs of the region, the stomach branches, and other organs. The first or ventral portion of the female duct (*v*), which is usually considered the vagina† or *bursa copulatrix* is rather thick, increasing in diameter to the anterior bend, and it receives, on each side, the ducts of very

* This preparation has been stained with borax-carmine and with picric acid, so that the organs show very plainly. The spermaries are dark brown and the ovaries bright red. Other mounted specimens of the species agree well with this one.

† See note under the genus, p. 477.

numerous "shell glands" (*w*) which radiate outward and backward from it. The outlines and connections of the large uterine sacs, which also join the vagina, cannot be distinctly seen, but they lie alongside the stomach, and are filled with eggs. The *vas deferens* also runs up along each side of the stomach as a convoluted tube.

Off Cape Cod, at Station 307, in 31 fathoms, 1879; also at Station 747, off Nantucket Shoals, in 13½ fathoms.

**Leptoplana variabilis** (Girard) Diesing.

*Polyscelis variabilis* Girard, Proc. Boston Soc. Nat. Hist., vol. iii, p. 251, 1850.

*Leptoplana variabilis* Diesing, Revision Turbell., Sitz. mathem-nat., xliv, p. 542. 1861.

PLATE XLI, FIGURE 7; PLATE XLIII, FIGURES 2, 2*a*, 3, 3*a*, 3*b*.

*Description of living specimens:*—Body thin, smooth, oblong or elliptical in extension, with the edges usually much undulated; very active and changeable in form.

Ocelli black and conspicuous; the cerebral clusters, which are situated over and in front of the ganglions, are decidedly elongated, usually fusiform and distinctly widest in the middle, but sometimes widest at the front end, subparallel or more or less divergent, and changeable in position according to the extension or contraction of the tissues about them; the hind end often extends as a narrow line or single row of ocelli back of the dorsal groups; the cerebral clusters may contain 25 or 30 ocelli each. The posterior or dorsal clusters are more or less circular or oval, each containing ten to fifteen rather large, conspicuous, black ocelli; a few of those in front are usually larger than the rest.

Color often yellowish brown, becoming paler toward the translucent margins and darker around the light gastric streak. The ganglions and nerves are not red.

Other specimens (as No. 736) are light salmon or light yellowish brown, thickly spotted with darker orange-brown, and with an interrupted pale streak over the stomach and reproductive organs.

Length of the larger specimens 12 to 18$^{mm}$; breadth 4 to 8$^{mm}$.

*Description of specimens mounted in balsam:*—The tissues are thin and fairly translucent. The pharynx has a large anterior, and a small short posterior lobe, with six principal lobes on each side. The mouth is nearly in the middle of the pharynx, but in advance of the middle of the body.

In the larger examples (Pl. XLIII, figs. 3 to 3*b*) the greater part of the body is filled with numerous ovarian and spermary vesicles, the

latter being more numerous and smaller than the former, and often grouped around them in clusters.*

The male genital orifice is far from the hind end of the body. The granular gland (*k*) is nearly round and prominent. The seminal vesicle (*r*) rests against the anterior side of the granular gland and consists of a central and two lateral parts, partially concealed by the granular gland, the exposed part being obovate or somewhat pyriform, as usually seen; the lateral portions are continuous with the *vasa deferentia* (*d*). The penis-sheath (*q*) is short, stout, somewhat conical; the penis itself, or verge, (*p*) is very long and slender, cirriform or hair-like, apparently somewhat chitinous, usually more or less coiled, often exsert in the preserved specimens.

The female genital orifice is usually surrounded by a broad circular pit. The first part of the duct (or vagina, *v*) is relatively large and long, a little expanded at the end, narrowing farther forward and bending upward and backward close to the male orifice, where it expands into a small ovate vesicle, from which it extends backward as a narrow moniliform tube (*v'*) which runs back beyond the external orifice and passes into the elongated cornucopia-shaped spermatheca (*s*), which is broad at the anterior, and pointed at the posterior end. In many specimens there appeared to be a small duct leading from the posterior end of the spermatheca to a minute external orifice (*x*), but this is uncertain, for it may be the nephridial duct.

Vineyard Sound to Eastport, Me., low-water mark to 42 fathoms or more. Abundant at Gloucester, Mass., on the shores, under stones, on the piles of wharves, and in tide-pools, especially at Ten Pound Island, 1878. Off Cape Ann, Mass., station 156, in 42 fathoms, muddy bottom, 1878 (No. 20). Casco Bay, Me., in tide-pools, 1873; Eastport, Me., in tide-pools and under stones at low-water mark, 1868, 1870, 1872. Vineyard Sound, on telegraph cable, off Cuttyhunk Island.

The original description of this species is almost useless for its identification. It is as follows: "This species is oblong-shaped, somewhat lanceolated, of a color varying from a light greenish yel-

* In the specimen (No. 20) illustrated (figs. 3–3*b*), the ovaries are colored light red, while the testes are colored dark red by the staining fluid used (alcoholic borax-carmine) and are, therefore, easily distinguishable. In order to prevent confusion, only a part of these organs are actually figured. The uterine sacs, oviducts, and most of the large sperm-ducts are also omitted, as well as the branches of the stomach, all of which can be seen, more or less distinctly, in the preparation.

low to an orange red, with a minute punctulation of a deeper red. The relative position of the eye-specks is subject to some variation. I have found it in Boston and Beverly harbors, always in deep water. It spawns in January and February. Entire length, half an inch."

The expression "deep-water" at the time the above was written probably meant 12 to 25 fathoms or less.

Although I have dredged extensively in the same waters where Girard's species was taken, and at all depths, I have never obtained any planarian that could be referred to his species with any probability of correctness, unless it be the species described above, which is a common one in Massachusetts Bay. There is nothing in Girard's description of specific importance except the color, which is more or less variable in all the species of the genus. Other species of *Leptoplana*, observed by me, are often tinged with reddish, or pale orange, but seldom so decidedly as his description would imply. Some of the varieties of the present species are, however, decidedly orange-red and have darker red or brown spots, nearly as stated in Girard's description. This fact, and the correspondence in locality induce me to adopt his name for this common species.

That his species was a *Leptoplana* is probable, because of his calling it a *Polycelis*, which, as used by authors at that time, was nearly equivalent to *Leptoplana*. From the description alone, it would, of course, be impossible to tell even the genus to which his species belongs, for no indication of the actual arrangement of the ocelli is given.

It is quite possible that this species is identical with *L. ellipsoides* Girard, described by him a few years later from the drawings of Dr. Stimpson. The latter, as it exists in the Bay of Fundy, is a much larger and broader form, with less conspicuous ocelli, and presents some other differences, as noted below. But these variations may be due to greater age, or to more favorable conditions of growth. My specimens are not sufficiently numerous to enable me to form a complete series between the two typical forms. They are, at any rate, very closely allied.

Among foreign species, the nearest relative of this species is, perhaps, *L. Dröbachiensis* (Œrsted) of Greenland. The latter has more numerous lobes to the pharynx and stomach, and differs, also, in the form of the genital organs.

## Leptoplana ellipsoides Girard.

*Leptoplana ellipsoides* Girard, in Stimpson, Invert. Grand Manan, p. 27, pl. 2, fig. 16. 1853; Diesing, Revision der Turbellarien, Abtheilung, Sitz. d. mathem.-naturw., xliv, p. 533, 1861; Lang, Polycladen, p. 512, copy of original description.

PLATE XL, FIGURES 5, 6; PLATE XLIII, FIGURES 4, 4*a*, 4*b*.

*Description of living specimens:*—Body large, flat, rather thin, usually broad-ovate or broad-elliptical, but capable of becoming long-elliptical; the edges are thin and frequently strongly undulated, and are capable of being used for swimming by means of rapid undulatory motions.

Ocelli are small, black, and not very conspicuous; those in the dorsal clusters are larger than those in the cerebral clusters. The latter are relatively rather small, somewhat elongated groups, broadest near the middle, narrowing to a point anteriorly, and containing numerous (often 30 to 40) minute crowded ocelli. They are situated a little forward of the other groups in usual states of the body, but in some states of contraction of the anterior and dorsal region they may be drawn back, so as to lie between, or even behind, the dorsal groups. The dorsal groups are usually nearly circular and contain numerous (20 to 25) ocelli of different sizes, some of which are much larger than the rest.

Color, various shades of dull yellowish brown, greenish brown, and reddish brown, usually more or less distinctly marked with irregular spots or blotches of darker brown, and with small specks of whitish; a lighter colored median streak runs over the gastric region, and another covers the genital region farther back; frequently the two streaks are united into a continuous median streak that extends nearly the whole length of the body. Under surface pale grayish or yellowish white, the whiter pharynx and *vasa deferentia* showing through indistinctly.

Length of large specimens, in extension, 25 to 35$^{mm}$; breadth 12 to 20$^{mm}$.

*Description of mounted specimens:*—The specimens mounted in balsam are not sufficiently translucent to show distinctly many of the internal organs, owing in part to their large size and the great amount of pigment, but more particularly to the fact that the body is so densely filled with ovarian and spermarian vesicles that the other organs are obscured.

The male genital organs (Pl. XLIII, figs. 4*a*, 4*b*) are close to the posterior end of the stomach; there is a conspicuous, nearly round, granular gland (*k*); at the anterior end of this, and partly concealed by it, the seminal vesicle (*r*) can be seen; its form is pyriform or rounded and cap-like, its length, as exposed, being usually greater than its breadth; it consists of a central, and two lateral parts, which are dilations of the *vas deferens;* the penis-sheath is stout-cylindrical or somewhat expanded at the end; the penis (*p*) is long and slender, cirriform, coiled up more or less in contraction; the external opening is often, in preserved specimens, raised on a conical elevation, and sometimes the penis sheath is protruded as a clavate papilla.

The female genital opening is usually situated in a broad funnel-shaped depression of the surface in preserved specimens; from this opening the nearly cylindrical, thick-walled, tubular duct or vagina (*v*) runs forward nearly or quite to the male orifice, where it bends upward and then turns backward upon itself in the shape of a siphon; at the bend it expands somewhat, into a small vesicle; but its distal or dorsal portion is a narrow, somewhat moniliform tube (*v'*), there being a series of slight constrictions along most of its length; it extends back beyond the external female orifice into a rather large, elongated, somewhat flask-shaped, thick-walled spermatheca (*s*). The first or ventral portion of the female duct (vagina, *v*) receives, on each side, numerous slender ducts of the tubular shell-glands that radiate outward from it in every direction, except directly forward. The connections of the uterine sacs or oviducts were not observed. Opposite the posterior end of the spermatheca there appears to be a minute external orifice that communicates by a slender duct with the spermatheca, but this connection was not fully demonstrated, nor was it seen in every specimen examined; it may be the nephridial duct.

Gulf of St. Lawrence to Casco Bay, low-water mark to 60 fathoms. Common at Eastport, Me., and Grand Menan, N. B., 1862 to 1872, at low-water mark under stones, in tide-pools, and at all depths down to 40 fathoms, on stony bottoms. Halifax, N. S., 8 to 10 fathoms, 1877. Casco Bay, Maine, 10 to 12 fathoms, 1873.

This species, as stated under *L. variabilis*, is closely related to the latter and may prove to be only a larger and more fully developed variety of it. The principal differences, externally, are the larger, broader, and more robust body, and the relatively smaller and much less conspicuous ocelli. The color is variable in each and not essentially different, unless the small white specks of the present species

be characteristic, which is doubtful. Internally, the structure is very similar, but the first part of the female duct (vagina) in this species is relatively shorter and smaller, and the spermatheca is somewhat swollen or flask-shaped, while that of *L. variabilis* is rather cornucopia-shaped. The male organs are very much alike.

The amount of the variations in form, proportions, and position of the genital organs that may be produced by the preserving and hardening fluids is, however, very uncertain. These closely allied forms should be more carefully compared as to these organs, when living, or at least before immersion in hardening agents.

## Leptoplana angusta, sp. nov.

PLATE XL, FIGURE 8; PLATE XLIV, FIGURES 2, 2*a*, 3.

Body very changeable, in extension rather narrow and elongated, elliptical or oblong, the length exceeding half an inch and equal to about three times the breadth, very thin, with the margins flexible, and usually more or less undulated and curled; the front end is usually rounded; the posterior end is often notched or emarginate in the middle.

The cerebral and dorsal clusters of ocelli blend, and form two nearly straight and parallel fusiform groups, often nearly linear in the small specimens, pointed at both ends, and situated well forward. In the larger specimens these groups have a distinctly wider portion behind the middle, composed of a small cluster of larger ocelli belonging to the dorsal groups, which are partially detached from the cerebral groups; but in the smaller specimens the dorsal ocelli can hardly be distinguished from the others, which mostly lie in two rows in each group.

The retracted pharynx is large, elongated, elliptical, with numerous short, nearly equal lobes along the sides, of which about twelve or thirteen pairs can usually be distinguished. The mouth is somewhat behind the center of the pharynx. The stomach has numerous lateral branches and a great number of terminal twigs which anastomose pretty freely distally.

The reproductive organs extend entirely around the pharynx and stomach, thus forming an opaque or dark colored elliptical zone. The *vasa deferentia* in the larger specimens are often conspicuous organs along the sides of the stomach. The uterine sacs in some specimens are large and swollen with masses of eggs, opposite and behind the posterior part of the stomach. The copulatory organs

are situated rather far back and are not easily seen in most of the preparations. The male organs consist of a large, elliptical, muscular penis-bulb more or less concealed by a large round granular gland; the sessile, rounded or oval seminal vesicle is situated above and partly behind the anterior end of the granular gland; the penis-sheath is rather long and large, cylindrical or sometimes elliptical; the penis is, apparently, simple and conical, without a slender chitinous cirrus. The glandular portion of the siphon-shaped female duct is elongated and dilated anteriorly, in the largest specimens, and extends from near the posterior margin of the body forward to, and often beyond, the male orifice, as seen in the best preserved specimens. This region is usually so altered by contraction that the reproductive organs are evidently much altered in form and position in nearly all cases, and therefore vary in different specimens from the same lot. The larger specimens are filled with numerous ovarian and spermarian folicles, and the uterine sacs and *vasa deferentia* are swollen with their contents.

Color above, while living, various shades of light brown, often tinged with darker brown in the middle and at the margins.

Length, in extension, while living, 12 to 16$^{mm}$, breadth 4 to 6$^{mm}$.

Provincetown, Mass., 1879, abundant among hydroids, barnacles, etc., on the bottom of a whaling vessel, recently arrived from off the Carolina coast. It was associated with several southern species of mollusks, crustaceans, etc.

This species is not a typical *Leptoplana*, but it appears to be closely allied to several foreign species referred to that genus by Lang and others. In its external characters it agrees with the genus *Elasmodes* of Stimpson, which Lang unites with *Leptoplana*.

**Trigonoporus** Lang, op. cit., p. 502.

Pharynx with numerous lateral lobes, stomach with about five or six pairs of main branches, which at first branch arborescently, but distally anastomose more or less completely.

Marginal ocelli numerous, but minute; many other similar minute ocelli are scattered over the frontal region. Cerebral ocelli numerous, forming large clusters over the ganglions and frontal nerves.

Dorsal ocelli small and numerous, forming crowded groups, sometimes confused with the cerebral clusters. Male copulatory organ with a well developed pyriform penis-bulb and granular gland combined; muscular penis-sheath conical or funnel-shaped; penis simple, conical; seminal vesicle apparently wanting. Female duct

siphon-shaped, with an external opening at each end. Its anterior opening is very near the male orifice (in contracted specimens often in the same depression); the posterior orifice is smaller and considerably farther back. A special spermatheca is wanting, or may be formed by a dilation at the anterior bend of the vagina, and not much specialized.

The single European species (*T. cephalophthalmus*), for which this genus was constituted, is a long and narrow form, with the groups of ocelli all confused, while our two species are broad and stout forms, with the four main clusters of ocelli clearly distinct, and arranged much as in *Leptoplana* and *Cryptocelis*, from which they differ but little in general appearance, when living. From *Leptoplana* they are, however, easily distinguished by the presence of numerous marginal and frontal ocelli, and by the peculiar structure of the genital organs. In the structure of the reproductive organs, and especially in having two orifices for the female duct, both of our species agree well with the type-species of *Trigonoporus*.

The cerebral ganglions and main nerve-trunks in both of our species are distinctly pale red in life, the color resembling that of the nervous system of certain nemerteans belonging to the genera *Lineus* and *Cerebratulus*. This observation was repeated many times, and in different years, so that it can scarcely be due to any temporary cause. I have not observed a red color in the nervous system of any other planarian.

### Trigonoporus folium Verrill.

*Leptoplana folium* Verrill, Marine Invert. of Vineyard Sound, etc., pp. 632 [338], 1873; Lang, op. cit., p. 512; copy of original description.

PLATE XLI, FIGURES 5, 5*a*, 6; PLATE XLII, FIGURES 5, 5*a*, 5*b*; PLATE XLIV, FIGURES 4, 4*a*, 4*b*, 4*c*, 4*d*, 5*b*, 6, 7.

*Description of living specimens*:—Body very changeable in form, rather flat, leaf-like, in extension oblong-elliptical or ovate, and usually narrowest anteriorly, capable of contracting to short, rounded or broad-ovate forms; margins more or less undulated, when in rapid motion.

Ocelli small and rather inconspicuous; the dorsal groups are situated at about the anterior fourth or fifth; the cerebral groups are elongated, irregularly fusiform, there being two broader portions, united by a narrower one, in the middle of each; they taper to a point at both ends, with numerous minute ocelli in the broader parts, near the middle and over the front and posterior ends of the gan-

glions; they are nearly parallel, not far apart, extending both behind and before the brain, but diminishing in breadth over the middle of the ganglions; one or two of the anterior ocelli are usually distinctly larger than most of those in the clusters. The smaller but more conspicuous dorsal groups, which are a little farther apart and situated rather behind the ganglions, contain a moderate number of rather larger ocelli, forming somewhat angular or irregularly rounded clusters, while just behind each group there is usually a small detached cluster of two, three, or more ocelli. In some specimens these small clusters are not noticeable, while in others they nearly blend with the main clusters.

The mounted specimens show, also, two, three or more rows of minute marginal ocelli, extending around the anterior margins, back to the middle of the sides, and in small numbers even to the posterior end; many other similar minute ocelli* are scattered over the whole frontal region, in advance of the cerebral groups, and some of the largest of these are ranged along the main frontal nerves.

Color, in life, yellowish flesh-color or pale ocher-yellow, lighter or darker yellowish brown, etc., becoming paler and translucent near the margins which are whitish; the ganglions and main nerves are pale red or pink; the stomach has very numerous, much lobed and divided branches, which often show through as brown markings; over the stomach and pharynx there is a whitish or pale reddish streak; behind the gastric streak there is a small elliptical whitish patch over the genital organs. The ventral surface is paler and shows a whitish gastric spot and the opaque *vas deferens* running up along each side of the stomach.

Length in life, 18 to 25$^{mm}$; breadth 10 to 15$^{mm}$.

*Description of specimens mounted in balsam:*—The ocelli, in the mounted specimens (Pl. XLIV, figs. 4*b*, 5*b*), appear more numerous than in the living ones, because more of those that are situated deep in the integument become visible, and consequently the shape of the clusters, especially the cerebral ones, appears to be different, in addition to the effect of contraction. The brain (fig. 5*b*) is only slightly bilobed. The stomach-branches are so thoroughly anastomosed that the body-parenchyma, as seen by translucency, appears to be vermiculated, or divided in many places into polygonal compartments, in some places even having a honey-combed appearance while in

* These small scattered and marginal ocelli were not noticed in the living specimens, doubtless owing to their small size. The examinations were mostly made with a good pocket lens, or a dissecting microscope.

other parts the spaces are rosette-like; owing to the contraction of the tissues the surface is reticulated by corresponding grooves. The ovaries and spermaries when present are numerous and arranged somewhat in rosettes, but they are not developed in most of the specimens, even when of large size.* The granular gland and muscular penis-bulb together form a conspicuous, broad-pyriform organ (Pl. XLIV, figs. 4, 4*a*, 6, 7, *k*), which is partially translucent and shows a radially vermiculated structure within (*k'*) and a central funnel-like opening to the ejaculatory duct; the anterior, opaque, rounded, glandular portion, forms, in some specimens, a slight median angle, or prominence. The muscular penis-sheath (figs. 6, 7, *q*) is strong and usually regularly funnel-shaped. The penis (*p*) is small, tapered, styliform.

The anterior female pore is near the male orifice, and in some specimens, owing to contraction of the tissues, both are brought close together into a single pit-like depression (figs. 4, 7); the dorsal portion of the female duct (*v'*) is somewhat elongated, and its posterior opening is at a considerable distance from the anterior orifice; the ventral part of the duct (*v*) is shorter and broader, and it receives the ducts of numerous shell-glands (*w*); at its upward bend it is considerably dilated, and this part probably serves as a spermatheca.

Behind the second female orifice (♀') there is usually visible a slender median duct which appears to terminate in a minute median pore (*u*); this is, perhaps, the central nephridial duct, but its extension forward could not be traced in the preparations.

The *vasa deferentia* are large and convoluted, in the specimens containing ova, and extend forward to or beyond the middle of the stomach. The uterine sacs, in the same specimens, are large and moniliform, each one containing four or five rounded masses of eggs; they extend forward to about opposite the mouth.

In the sexually developed specimens the spermaries are very numerous, especially between the outer meshes of the gastric branches, where there are few ovaries; farther toward the center they often form groups of five to ten around a single ovarian folicle, thus having the appearance of a rosette, with the larger ovarian folicle in the center, each rosette occupying one of the meshes formed by the digestive tubes.

Long Island Sound to Eastport, Maine, from low-water mark to

* Probably the breeding season was mostly past when they were collected, in midsummer, but they are present in the original specimen obtained in April, and also in the one from station 784.

54 fathoms. Off Watch Hill, R. I., 4 to 6 fath. among algæ, specimen with ova, April, 1872 (A. E. V.); off Point Judith, R. I., sta. 784, in 20 fath., with ova, 1880; off Block Island, sta. 812, 28½ fath.; off Gay Head, Martha's Vineyard, 18 fath., 1887; off Cape Cod, sta. 301, 27 fath., 1879; off Cape Ann, Mass., at stations 134 and 136, in 26 fath., and at sta. 182, in 45 fath., 1878; Eastport, Maine, 1870.

There is considerable variation, both in external appearance and in the form of the reproductive organs in this species, but I believe the differences observed are due to age, season of the year, presence or absence of ova, and the amount of contraction when preserved. As it might be doubted whether all the forms can be referred to *T. folium*, I have thought it well to give here a special description of the original type-specimen of that species, for comparison with the later and better specimens, described above.

*Description of the original specimen from life:*—Body very flat, foliaceous, with the margin thin and undulated; outline very changeable, broad-ovate, narrowing to an obtuse point at the anterior end; sometimes oblong or elliptical and but little narrowed anteriorly.

Ocelli in four groups, near the anterior end; the anterior or cerebral clusters are parallel, narrow, elongated, widest in the middle, close together, almost blending on the median line, and composed of many very minute ocelli, less easily seen than those of the other clusters; the terminal ocelli are largest; the dorsal or posterior groups are rather small and irregular; in some states of extension often triangular, with the pointed end backward; marginal ocelli numerous, but minute, in two or three rows near the edge, with others scattered over the frontal region, visible only when much magnified.

Color, pale yellowish flesh-color, veined with dendritic streaks of darker flesh-color and whitish lines; an interrupted longitudinal whitish streak in the middle, over the stomach and pharynx, and a small median whitish streak farther back.

Length, $20^{mm}$ to $25^{mm}$; breadth, $10^{mm}$ to $15^{mm}$.

Off Watch Hill, 4 to 6 fathoms, among rocks and algæ, April, 1872.

*Description of the original type as preserved:*—This original type-specimen, mounted in balsam, shows many of the anatomical characters (Pl. XLIV, figs. 4–4*c*), but the posterior end had been mutilated in life and only partly healed; in consequence of this the female reproductive organs are not perfect. This also accounts for the broad emargination of the posterior end, mentioned in the original description.

The pharynx is short and broad and has five large lobes on each side, some of which are bilobed, and two small lobes near the anterior end. The mouth is somewhat behind the middle of the pharynx. The stomach has five or six main branches on each side and these are much branched and extensively anastomosed distally, as described above.

The meshes or interspaces between the stomach-branches are filled with large numbers of rather large ovarian folicles and a much greater number of smaller spermaries which are grouped closely around each of the ovarian folicles, so as to form rosettes where they are not too much crowded; distally the spermaries increase in numbers, while the ovaries diminish.

The combined granular gland and penis-bulb (figs. 4, 4*a*, *k*) form a regularly pyriform organ, rounded anteriorly, and having essentially the same structures as in the specimens described above; the penis and penis-sheath (*q*) also agree well with those already described. Owing to the mutilation of the female organs and the contraction of the parts, the anterior female orifice and the male orifice are brought close together, and the posterior female orifice is not distinguishable.

This is a very active and restless species. One specimen protruded its multilobed pharynx when placed in alcohol. A specimen (sta. 136, July 26, 1878) was filled with white eggs, visible through the integument of the ventral side, while living.

When preserved in alcohol, the specimens retain their form better than those of most of the related species, and usually do not curl up much; the preserved specimens are rather thick, firm, usually broad-ovate, narrower toward the front end, which is often somewhat lanceolate; the mouth is usually visible as a small round central opening; the small male reproductive orifice, or a papilla in its place, is often distinct.

### Trigonoporus dendriticus, sp. nov.

PLATE XLI, FIGURE 4; PLATE XLII, FIGURES 4, 4*a*, 4*b*; PLATE XLIII, FIGURE 5.

Body usually ovate with the anterior end narrowed and more or less pointed, edges undulated and flexible, but the form is subject to considerable changes, though less so than in the species of *Leptoplana*.

Ocelli minute and rather inconspicuous; the cerebral groups are near together, irregular and somewhat elongated, composed of numerous very small ocelli which mostly lie over the anterior and

the posterior ends of the ganglions, so that each cluster is constricted in the middle, or somewhat hour-glass shaped; the dorsal groups are more conspicuous, but usually smaller, irregular, longer than broad, often somewhat elongate-triangular, with the acute end directed backward;* they are well separated from the cerebral groups and are, for the most part, behind them; marginal ocelli are small but numerous, in two or three rows extending back to the middle, while numerous small ocelli are scattered over the whole frontal region.

Color pale yellowish or pinkish, with brown dendritic markings, due to the much divided and lobed stomach branches; the pharynx, showing distinctly through the integument, forms a deeply lobed central spot. The cerebral ganglions and nerves are reddish. The mouth is rather large, situated in front of the middle, and about opposite the second pair of large pharyngeal lobes. Pharynx is large and has about five pairs of large and long lobes. The main branches of the stomach fork and divide arborescently at first, and the branches are much lobulated along their sides, giving them a fern-like appearance. They do not anastomose so freely as those of *T. folium.*

Length 12 to 15$^{mm}$; breadth 6 to 8$^{mm}$.

The reproductive organs (Pl. XLIV, fig. 3) agree pretty nearly with those of *T. folium*, as seen in the mounted specimen. The penis-bulb and granular gland together are more oblong than in that species, and there appears to be a constriction near the front end, partially separating the cap-like anterior portion from the rest. The penis is short, conical, and simple; its sheath is not so thick and strong as in *T. folium.* The anterior female orifice is a little distance back of the male orifice; the glandular part of the vagina is elongated, and its posterior orifice is situated well back.

Off Race Point, Cape Cod, sta. 317, in 25 fathoms, 1879.

**Discocelis** Ehrenberg, 1832, (emend.); Lang, op. cit., p. 466.

Body broad, usually ovate, foliaceous, marginal ocelli present, cerebral and dorsal ocelli variable in number, usually in four groups. Mouth central or a little in advance of the center. Pharynx large, and sometimes with divided accessory pouches. A single common

* The mounted specimens, as well as some of my sketches from life, show that all the groups of ocelli as represented in fig. 4, Plate XLI, are too regularly triangular, and the posterior groups are too large and too elongated; the enlarged figure on Plate XLII. fig. 4*a*, is from the same specimen, after mounting; but doubtless the shape of the groups is more or less altered by contraction.

genital opening. Female genital organ with a glandular accessory vesicle.

The following species is referred to this genus chiefly on account of its external resemblance to the typical species. I have had no mature specimens sufficiently well preserved to enable me to study the reproductive organs.

### Discocelis mutabilis Verrill.

*Polycelis mutabilis* Verrill, Report on the Inverteb. of Vineyard Sound, etc., p. 746, (452,) 1873.

Lang, op. cit., p. 616, copy of original description.

PLATE XL, FIGURE 7; PLATE XLII, FIGURES 6, 6*a*, 7.

*Description of the original specimen from life:*—Body much depressed, thin, changeable in form, often elliptical or oval, frequently broad and emarginate in front, and tapered posteriorly. Marginal ocelli very distinct, black, forming several rows along the front border, but only one or two rows laterally. Dorsal and cerebral ocelli larger, forming three pairs of rather ill-defined clusters; the outer or dorsal clusters are largest, often convergent backward; a pair of smaller cerebral clusters is situated a little in advance, and nearer together; the third pair is a little farther forward and still closer together, often more or less confused with those next behind them.

Color, yellowish brown, darker centrally; or pale yellowish, thickly specked with yellowish brown. Length, about 7 to $9^{mm}$; breadth, 5 to $6^{mm}$.

Thimble Islands, near New Haven, Conn., 1 to 2 fathoms, among red algæ, 1872.

The original type-specimen, described above, was mounted in balsam for the microscope, when first obtained. It is still in my possession, and from it the figures (Pl. XLII, figs. 6, 6*a*) have been drawn with the camera-lucida. Unfortunately the internal organs are not well preserved. The reproductive organs cannot be seen at all. Possibly the specimen was immature, with imperfectly developed sexual organs, but its size would indicate that it was adult.

The pharynx and stomach are large and have numerous lateral obes. As near as can be made out, the elongated stomach has eight or nine principal branches on each side, but they are indistinct, owing to the poor state of preservation of the specimen.

The marginal ocelli are rather conspicuous and extend back nearly as far as the posterior end of the stomach. The four groups of

cerebral and frontal ocelli are more or less confused in the preparation, and are unusually far apart, doubtless owing to compression in mounting the specimen; the distortion of the groups is doubtless due to the same cause; behind each cerebral group there is a rather large isolated ocellus. The posterior or dorsal groups are conspicuous, a little elongated, elliptical or oval, somewhat pointed behind; each is composed of about 12 black ocelli, several of which are considerably larger than the others, reniform in shape, with a transparent, lens-like portion on the concave side.

I have never found another large specimen that can be referred to this species.

*Description of young specimens:*—Several very young individuals were taken at the surface, with towing nets, both at Newport, R. I., and at Wood's Holl, Mass., which apparently belong to this species, but they are all too immature to be identified with certainty, until intermediate sizes shall have been observed.

One of these young specimens, figured from life, is represented on Plate XL, figure 7, in its contracted form; another unpublished figure of the same individual is considerable longer with the frontal ocelli farther forward, and with the stomach longer and showing eight or nine pairs of lateral branches. The reproductive organs are not distinctly developed. The form is generally obovate or obcordate, with a distinct emargination in front. The ocelli form six groups; the frontal groups contain each only one or two ocelli; the cerebral groups contain each about three ocelli in a single row; each of the outer or dorsal groups contains three ocelli. (Pl. XLII, fig. 7).

The color is translucent whitish with delicate, pale yellow dendritic marking and a deeper yellow gastric spot; the transparent margin is elegantly marked with a row of light golden yellow spots. It is active and graceful in its movements.

This specimen was taken at the surface of the sea at Wood's Holl, Aug. 16, 1882.

### Tribe, COTYLEA Lang.

Acetabulum ventral, behind the female opening.* Mouth and pharynx situated between the middle and anterior end of the body.

* The existence of a ventral acetabulum or sucker is not strictly diagnostic of this group, for certain species of *Leptoplana* and of *Planoceridæ* have, also, a ventral sucker of the same kind. But in all the latter groups, so far as I know, the sucker, when present, is in front of the female orifice. Perhaps the pit-like depression around the female orifice of certain species of *Leptoplanidæ* is of the same nature and for the same purpose.

Pharynx tubular, long or short, exsertible. Main stomach cavity not extending forward beyond the pharynx. Lateral branches of the stomach variable in number, reticulated or dendritic.

Tentacles, when present, situated at the anterior margin, often wanting. Ocelli numerous, usually forming two cerebral clusters; an anterior marginal row; and clusters in the tentacles, when these are present. Female organs are without a copulatory pouch and accessory vesicle or spermatheca. The male organs are variously situated, often anterior; sometimes they are paired, or even (in the genus *Anonymus*) multiple in a lateral row along each side. Development with metamorphosis.

### Family, EURYLEPTIDÆ Stimp., 1857 (emend.).

*Euryleptidæ* Lang, Die Polycladen des Golfes von Neapel, p. 553, 1884.

Body ovate or elliptical. Marginal tentacles present or absent. Mouth near the anterior extremity of the body. Pharynx tubular. Stomach long, narrow; its branches either simple or anastomosing. Ocelli are present at the front margin of the body and in the tentacles, when these are present; others form two cerebral groups.

### Eurylepta (Ehrenberg) Lang.

Body not papillose. Marginal tentacles well developed, elongated, not pliciform. Pharynx cylindrical. Principal lateral branches of the stomach about five on each side, not anastomosing. Male aperture under the posterior end of the pharyngeal sac.

### Eurylepta maculosa Verrill, sp. nov.

PLATE XLI, FIGURES 2, 3.

*Description of living specimens:*—Body thin, very changeable, usually elliptical or oblong, and more or less elongated, with the margin very thin; in motion often wavy and undulated, and constantly changing its form; frontal margin rather narrow with two moderately long, obtuse, flattened marginal tentacles, which are flexible and changeable in form.

Ocelli numerous; on the lower and anterior sides of the tentacles there are clusters of numerous, minute, black ocelli, but they do not extend much above the middle of the tentacles; numerous similar marginal ocelli are scattered between the tentacles on and near the front margin; back of the tentacles, the distance varying with the state of contraction or extension, there are, above the ganglions, two elongated groups of cerebral ocelli, side by side, and so close together as to appear, at times, like one broad group; each of these groups

consists of twelve or more ocelli, of which one, near the middle in each group, is larger than the rest.

Color pale, translucent, yellowish or pinkish white, irregularly specked and mottled, or veined, with purplish or brown, and usually with a number of darker and more distinct, small, brown spots scattered over the central area, and especially along the middle of the back, while around the margin the color markings form distinct radial spots.

Length 10 to 12$^{mm}$; breadth 6 to 8$^{mm}$.

Wood's Holl, on piles, July 14, 1881; in mud, Aug. 2, 1882; Naushon I., near Wood's Holl, Mass., at low-water mark, among algæ, Aug. 20, 1887.

This species has been met with but few times, and in each case only a single specimen has been obtained. It must be considered very rare on our coast. None of the specimens are sufficiently well preserved to allow any anatomical study.

### Family, Prosthiostomidæ Lang, p. 594.

**Prosthiostomum** Quatrefages, op. cit., p. 133, 1845; Lang, op. cit., p. 594.

Body elongated, smooth. No tentacles. Ocelli in cerebral groups and around the front margin. Mouth anterior, below the brain. Pharynx long, tubular, directed forward. Stomach elongated, with numerous lateral branches, not anastomosed.

Male genital organs behind the pharyngeal sac; two accessory seminal vesicles; penis uncinate, directed backward.

This is the only genus of the family.

**Prosthiostomum gracile** Girard.

*Prosthiostomum gracile* Girard, Proc. Boston Soc. Nat. Hist., vol. iii, p. 251. 1850.
*Elasmodes? gracilis* Stimpson, Prodromus, p. [3] 21, 1857.

Woodcut, Fig. 1.

The original description of this species is so meager that no one has hitherto been able to identify it, so far as I know.

It reads as follows: "It differs from other species of the same genus by its very slender body and the arrangement of the eye-specks, which are disposed in four groups; of which the first and second are in a single pair, the third triple, and the fourth double. From Boston Harbor."

The generic position has been doubtful. Dr. Stimpson, who states that he had Girard's original drawings of planarians for examination, refers it doubtfully to *Elasmodes* (= *Leptoplana* in part).

The only specimens of the genus *Prosthiostomum* that I have found on the New England coast are quite young. Presuming that Girard's species was correctly referred to this genus, my young specimens may, very likely, belong to the same species, for the ocelli have a similar arrangement.

Fig. 1. *Prosthiostomum gracile.* Young, × 8.

When living the body is long-elliptical in extension (see cut 1, from life) and quite thin and translucent. The stomach has about fourteen main branches on each side, and these are much divided distally. Those of the anterior pair curve outward and forward around the pharyngeal region and ocelli. The tubular pharynx was only imperfectly seen, and was probably not fully developed. The cerebral ocelli form an interrupted curved row of about four on each side, of which the anterior pair is largest. The posterior or dorsal clusters are each represented by two ocelli, placed obliquely. The marginal ocelli are very distinct, and form a single row around the front edge. The reproductive organs were not developed.

Color pale yellowish white with darker yellow arborescent markings, due to the gastric branches. Other specimens are light yellowish green, with a whitish gastric spot and pale radial lines, and a greenish median line posteriorly.

Length of the largest about $4^{mm}$; breadth $1{\cdot}25^{mm}$.

Noank, Conn., July 9, 1874; Wood's Holl, Mass., July 25, 1881; New Haven, Conn., Oct. 14, 1892. Boston Harbor (Girard).

It is probable that Girard's specimens were also immature, and perhaps but little larger than those here described. He does not give the size.

## MONOGONOPORA or TRICLADIDEA.

Body generally narrower and relatively longer than in the *Digonopora.* Sometimes a distinct muscular ventral foot is present. Sometimes a posterior sucker is developed. In some genera there are dorsal tentacles; in others, frontal lobes. The mouth is generally somewhat behind the middle. The pharynx is generally simple*

* In the American fresh-water genus *Phagocata* (Leidy), besides the central pharynx, there is a row of smaller ones along each side, each one arising from the base of a lateral secondary stomach-branch.

and tubular, often cylindrical or urn-shaped, and capable of being protruded fully from the mouth; when retracted it lies in the median line, in a definite pharyngeal cavity. Its base connects with the stomach at the central part from which the three main gastric branches arise.

Two of the main gastric branches run backward, one on each side of the pharyngeal and reproductive regions, and from these numerous smaller transverse branches, either simple or branched, run out toward the margins, and still smaller transverse branches often run inward toward the center. The anterior main branch is median and gives off lateral branches more or less symmetrically on each side.

The brain, frontal nerves, and main lateral nerve-trunks are generally well developed; a small marginal nerve and a complex nervous plexus can often be distinguished without difficulty.

The ocelli are often only two, situated over or near the cerebral ganglions, in other cases three pairs, and in many genera they are numerous, in two groups; sometimes they are lacking. A median otocyst is sometimes present.

The species are nearly all hermaphrodites. There is one common genital orifice, posteriorly situated. The ovaries, or *germaria*, are usually two, anteriorly situated, and special *vitellaria* are developed along the sides. Spermaries are generally, but not always, numerous and scattered. The copulatory organs present many variations. The eggs are generally enclosed in capsules. Some species are viviparous. Development is direct.

Most of the species of this group inhabit fresh-water and moist places on the land, but there are several marine genera. The terrestrial species are found chiefly in warm and moist countries, but a single, small terrestrial species (*Rhynchodemus sylvaticus* Leidy) inhabits New England, as far north as New Haven, where I have often observed it.

## Family, Bdellouridæ Verrill.

*Bdelluridea* Diesing, op. cit., p. 518, 1861.

Body elongated, flattened, highly muscular, leech-like, with a well developed posterior acetabulum or sucker. Brain and lateral nerve-trunks well developed; marginal nerve distinct. Pharynx plicated, cylindrical in extension. Two posterior main gastric branches not united posteriorly. Ectoparasites.

This family is constituted for the following genus only.

**Bdelloura** Leidy.

Proc. Acad. Nat. Sci. Philad., vol. v. 242, 1851; Stimpson, Prodromus, p. 6, 1857.
*Bdellura* Diesing, Sitzb. der mathem-naturw., Abtheilung, Dendrocœlen, p. 518, 1861.

Body flat, lanceolate, with thin muscular edges along the middle, adapted for swimming. Acetabulum nearly as wide as the body, separated by a constriction. Mouth behind the middle. Tentacles none. Ocelli two, reniform, with a front lens. Brain large, bilobed, with several pairs of frontal nerves; lateral nerve-trunks (Pl. XLIV, fig. 8, *n*, *n'*,) large, united by a posterior commissure in the acetabulum, and by others, farther forward, behind the genital orifice. Lateral gastric branches (fig. 8, *g'*) more or less divided. Penis simple, conical, unarmed (figs. 8, 8*a*, *p*). A pair of female, accessory, lobulated glandular organs or "uterine sacs" (fig. 8, *x*) is situated about opposite the genital pore. Unicellular mucus-glands are present. Rhabdites are wanting. Eggs are enclosed in capsules.

The only known species is an active ectoparasite on *Limulus*, but it is able to swim freely, with an undulatory, leech-like motion.

**Bdelloura candida** Girard.

*Vortex candida* Girard, Proceedings Boston Soc. Nat. Hist., vol. iii, p. 264, 1851.
*Bdelloura parasitica* Leidy, Proceedings Acad. Nat. Sci. Philad., vol. v, p. 242, 1852; Stimpson, Prodromus, p. 6, 1857.
*Bdelloura candida* Girard, op. cit., vol. iv, p. 211, 1852.
*Bdellura parasitica* Diesing. op. cit., p. 518.
*Bdelloura candida* Verrill, Rep. Marine Invert. Vineyard Sound, pp. 634 [340], 460 [166], 1873: Gissler, Amer. Nat., vol. xvi, pp. 52, 53, 1882 (egg-cases and young); Ryder, op. cit., pp. 48–51; 142, 143, 1882 (10 woodcuts of egg-cases, etc.; fig. 8 is reversed, head for tail), three species supposed to exist on *Limulus*.
*Planaria limuli* Graff, Zool. Anzeig., pp. 202–205, 1879; Woodruff, Bull. Mus. Comp. Zool., xxi, p. 19, 1891.

PLATE XL, FIGURES 10, 10*b*; PLATE XLI, FIGURE 8; PLATE XLIII, FIGURE 11; PLATE XLIV, FIGURES 8, 8*a*, 8*b*.

Body smooth and firm; muscular system highly developed; very changeable in form and very active; usually lanceolate, or broadest in the middle with the anterior end pointed. While adhering by the posterior sucker it can extend itself in every direction, at times

Fig. 2. *Bdelloura candida.* Adult, slightly enlarged.

becoming almost linear and looking very much like a small leech (*Clepsine*), but in contraction it becomes short-elliptical, oval, or oblong; the margins are thin and often undulated in life. Posterior

sucker well-developed, variable in form according to the state of contraction. It is able to swim rapidly with undulatory motions like those of a leech.

Ocelli two, black, conspicuous, near together, not far from the front end. Pharynx conspicuous in life, as a white median, cylindrical, sulcated organ showing through the integument; its open end is divided into about six small lobes. Mouth nearly central, or a little behind the middle. Transverse branches of the intestine numerous and crowded, about twenty-five to thirty principal ones on each side; mostly forked and lobulated distally, in adult specimens. The ovaries or germaria appear as somewhat opaque, whitish, round or pyriform organs near the bases of the fourth pair of branches of the stomach, and often about midway between the brain and the base of the pharynx.

Color whitish, grayish, or yellowish; the intestinal branches usually forming a central darker brown region, in the middle of which the pharynx forms an oblong white spot. The color of the gastric or intestinal branches varies according to their contents.

Length 15 to 25$^{mm}$; breadth 4 to 6$^{mm}$.

Cape Hatteras, N. C., to Casco Bay, Maine. Very common on the gills and gill-plates and other parts of the "horse-shoe crab," *Limulus polyphemus.*

The egg-capsules* are often found in large numbers attached to the gill-plates of *Limulus.* They are chitinous, brownish or yellowish, variable in size and form, usually oval, elliptical, or oblong, with the upper side convex and the under side nearly flat; the larger ones are about 3$^{mm}$ long and half as wide. Pl. XLIV, figure 8*b*. They are usually attached by a pedicle at one end, but sometimes at both ends. They contain from one to eight eggs or embryos, most commonly four to six. I have observed fresh capsules during the whole summer, and have seen them forming in October. showing that the breeding season is long. Pl. XLIII, fig. 11, *ca.*

It seems to me certain that the embryos formerly described and

* Dr. J. A. Ryder has described and figured three forms of these egg-capsules from the gills of *Limulus* (Amer. Naturalist, vol. 16, pp. 48–51) and thinks that they probably belong to distinct species. I have never found but one species of adult planarians on *Limulus.* In the case of one of the worms (fig. 8), supposed by him to be a distinct species, he mistook the caudal sucker for the head, and overlooked the eyes. Making this correction, the differences noted by him disappear. The differences in the capsules appear to be only variations of form and size of no great importance due. in part at least, to the age and size of the individuals producing them. The capsules produced by the largest specimens contain more ova than those belonging to young individuals.

figured by Mr. A. Agassiz,† under the name of *Planaria angulata*, did not belong to this species, for he stated that the eggs found by him were in "a string of eggs, mistaken at first for those of some naked mollusk," which is the case with the eggs of many Polycladidea, but not with those of this genus. The embryos observed by him were not sufficiently advanced for identification, but the structure of the stomach and its branches, so far as developed, agrees better with one of the Polycladidea than with that of any of the Tricladidea. The embryos of *Bdelloura* before leaving the capsules usually have the three main divisions of the stomach clearly visible, as well as the two eyes.*

This is a true parasite. It sucks the blood of its host and destroys the substance of the gills. When large numbers are present the gills of the *Limulus* are often extensively damaged in this way.

It is gregarious in its habits. Large clusters of the adults are often found grouped together on the joints of the legs as well as on the gills of *Limulus*. Even in confinement they preserve the same habit, adhering to the glass in large groups. When disturbed they often swim away with undulatory motions, like certain leeches.

This species is particularly favorable for anatomical studies, for the tissues are well differentiated and the various organs are more distinct than in many other allied planarians. The small amount of pigment in the integument is also favorable, especially when examined in the living or fresh condition. It is also very tenacious of life, for it will live for a long time without food in a very small amount of sea-water.

Some of the principal anatomical characters that can be made out with living specimens are represented on plate XLIV, figs. 8, 8*a*. Figure 8 shows a ventral view of the entire worm only slightly compressed, and figure 8*a* shows the posterior portion of another individual more strongly compressed, so as to show the reproductive organs better, but in this figure the intestinal branches (*g'*) are so much compressed that they appear unnaturally broad and swollen, while the outline of the posterior sucker is obscured, for the same reason. In this figure the nervous system is omitted.

The lateral gastric branches (left unshaded in fig. 8) in adult specimens are very numerous, ten to twelve arising from each side of the anterior trunk or median division of the stomach (*g*), while

* Several writers, have carelessly referred to the embryonic forms, described by Mr. Agassiz, as the young of this species, although the true egg-capsules of the latter have long been known.

† Annals Lyceum Nat. Hist., New York, vol. viii, pp. 306–309, 1866.

twelve to eighteen arise from each of the posterier divisions (*g'*). Those in the middle part of the body are mostly two-lobed or forked, and some of them are divided into three or four branches but some remain simple, especially toward the ends of the body. Under pressure all the branches usually appear lobulated along their sides and at their ends. The posterior trunks also give off from their inner borders smaller lateral branches which run in toward the median line, where those of opposite sides anastomose, back of the pharyngeal region. The two posterior trunks do not anastomose at their posterior ends in any of the examples studied by me. The anterior trunk terminates behind the brain.

The pharynx (*f*) is large, cylindrical or urn-shaped, according to its state of contraction, lobed or scolloped at the end, and often plicate or grooved along the sides. It is often protruded in alcoholic specimens.

The bilobed brain (*c*) is large and easily seen. Each lobe gives off from its antero-lateral and front borders about five main nerves (*c'*) which subdivide and run to the margins of the head. The main lateral nerve-trunks (*n*, *n'*) are large and distinct. They run back and anastomose directly in the posterior sucker. They give off, all along their course, lateral nerves that subdivide into many small branches, part of which go to the adjacent organs, while some go to and join the small but distinct marginal nerve (*n''*), in the vicinity of which they form a fine network. The principal transverse branches from the large nerve-trunks correspond nearly in number with the gastric branches. Back of the genital organs five or six, or more, transverse commissures unite the main nervous trunks. The eyes are well developed, with a blackish, reniform, pigmented retinal body and a frontal lens-like structure.

The genital orifice (♀, ♂) is situated just back of the pharyngeal region. The *vasa deferentia* (*d*) run alongside of the pharyngeal region as convoluted tubes; near the penis they become enlarged and serve as two seminal vesicles (*r*). The penis-bulb is pyriform or obconic, and the penis (*p*) is simple, styliform. The oviducts run forward from the female genital duct or vagina (*v*) on each side to the germaria or ovaries (*o*, fig. 8), which are situated well forward at or near the bases of the third and fourth pairs of gastric branches. The vitellaria or yolk-glands are very numerous; they are clustered around and between the gastric branches, together with the testes or spermarian folicles (*t*, *t*), which are smaller and more numerous.

Two rather conspicuous glandular organs or "uterine sacs" ($x$) are situated to the right and left of the genital orifice. Each one is connected with the genital duct by a convoluted tube. The function of these organs was not clearly ascertained, but they probably are of the same nature as the so-called "uterine sac" of other tricladial planarians, which serves in part as a spermatheca or seminal receptacle and in part as a shell-gland. In this genus their principal function is probably the secretion of the materials for the formation of the strong, chitinous egg-capsules.

## Family, PLANARIDÆ.

Body depressed, more or less elongated, often oblong or long-lanceolate in extension. Head sometimes broader than the body, often with its lateral or antero-lateral margins a little produced into points or angles, or somewhat auriculate. Tentacles sometimes present, usually absent.

Ocelli commonly a single anterior pair, with a frontal lens-like portion and a reniform pigmented body; sometimes three or more pairs; sometimes absent.

Pharynx single,* tubular, usually cylindrical; mouth central or subcentral. Lateral intestinal branches simple or dichotomously divided.

Eggs commonly laid in capsules. Some species are viviparous. Fluviatile and marine.

### Fovia Girard; Stimpson, emend., 1857.

*Fovia* Girard, Proc. Boston Soc. Nat. Hist., iv, p. 211, 1852; Stimpson, Prodromus, p. vi, 1857; Diesing, op. cit., Dendroc., p. 501, 1862; Jensen, Turbellaria ad Litora Norvegiæ. p. 74, 1878.

Body depressed, oblong or long-elliptical in extension, changeable; back a little convex, ventral side flat, the posterior part most muscular. Front of head often slightly produced and angular; sides of head rounded, at other times a little prominent or angular.

Ocelli two, anterior, rather large, with front lens and reniform pigmented body, each usually surrounded by a pale spot.

* The fluviatile genus *Phagocata* Leidy is here excluded from this family. It should be the type of a special family (*Phagocatidæ*) characterized by the presence of numerous secondary lateral pharynges, besides the median central one, and by several other peculiarities of structure. (See detailed description of its anatomy and histology by W. M. Woodworth, in Bulletin Museum Comp. Zool., vol. xxi, pp. 1–44, pl. I–IV, 1891.)

Mouth near or behind the middle. Largest lateral branches of the stomach usually more or less lobed or forked in the adult, simple in the young. Pharynx large, cylindrical. Reproductive organs not fully known. Penis conical, simple.

This genus appears to be closely related to *Gunda.*

**Fovia affinis** (Œrsted) Stimp.

*Planaria affinis* Œsrted, Entw. syst. Einth. Plattwürmer, p. 54.

*Fovia affinis* Stimp., Prod., p. [6] 24, 1857; Diesing, op. cit., p. 502, 1861; Jensen. op. cit., p. 74 (descr.)

? *Vortex Warreni* Girard. Proc. Boston Soc. Nat. Hist., vol. iii, pp. 264, 363, 1851.

? *Fovia Warreni* Girard, op. cit., vol. iv, p. 211, 1852; Stimp. Prod., p. [6] 24, 1857: Diesing, op. cit., p. 229, 1862.

*Planaria grisea* Verrill, Rep. Invert. Vineyard Sound, etc., pp. 633 [399]. 487 [193], 1873.

*Fovia grisea* Verrill, Check List Marine Invert., p. 13, 1879.

*Fovia littoralis* Verrill, op. cit., p. 13, 1879, (? *non* Müller sp.).

PLATE XLI, FIGURES 9, 9*a*, 9*b*.

Body very versatile, in usual extension oblong, widest behind the middle, round or subtruncate at both ends, or with the middle of the front margin a little prominent, sometimes contracting into broad-ovate or cordate forms. The antero-lateral angles of the head are often slightly prominent, subangular or rounded, and very mobile. Mouth nearly central. Principal lateral branches of the stomach mostly bilobed, others simple. The posterior part of the lower surface seems to be capable of being used to some extent as a sucker, for the creature often moves with quick jerks, like a leech, but it is not separated by any constriction from the rest of the body.

Ocelli two, not far apart, black, reniform, with a transparent front lens, each situated in a reniform white patch or spot, widest outwardly.

Color above, yellowish, grayish, reddish, or fulvous brown, or dark brown; margins and lower side paler; over the pharynx and stomach is a long, pale blotch in the middle above, extending in some examples the whole length of the body; the long cylindrical pharynx can sometimes be seen through the integument, especially beneath, extending in partly contracted specimens nearly to the posterior end. Plate XLI, figure 9*b*.

Length 4 to 12$^{mm}$; breadth 2 to 3$^{mm}$, in extension.

Ten Pound I., Gloucester, Mass., in tide-pools at low-water, and among eel-grass (*Zostera*) in the harbor, 1878; Casco Bay, Me., at low-water, under stones, 1873, (var. *grisea*); Watch Hill, R. I.,

at low-water mark, April, 1872 (A. E. V.), var. *grisea*. Beverly, Mass. (Girard), var. *Warreni*.

This species is not abundant on our coast. I have taken only a single specimen of a dark green variety (referred doubtfully to this species) at Eastport, Me., and Grand Menan, N. B., during many seasons spent in studying the fauna of that region.

Our species appears to vary widely in color, but I am unable to find other differences sufficient to indicate more than one species. Nevertheless, it may be well to designate the principal color-variations as varieties until better known, especially as they have already received specific names.

Variety, **Warreni** Girard.*

Color red or reddish brown.

Variety, **grisea** Verrill.

*Planaria grisea* Verrill, Invert. Vineyard Sd., p. 633 [339], 1873.

Color grayish, yellowish or greenish above, with a median whitish streak. Pl. XLI, figs. 9–9*b*.

The original description is as follows:—

"Body elongated and usually oblong in extension, often long oval or somewhat elliptical, obtusely pointed or rounded posteriorly; head subtruncate in front, often a little prominent in the middle; the angles are somewhat prominent, but not elongated. Ocelli two, black, each surrounded by a reniform white spot. Color yellowish green or grayish, with a central whitish stripe in the middle of the back, surrounded by darker; head margined with whitish. Length, in extension, 12$^{mm}$; breadth, 3$^{mm}$."

Watch Hill, R. I., under stones, between tides (April, 1872).

This species is referred to that of northern Europe with some doubt, owing to our imperfect knowledge of the internal anatomy of both forms. Externally they appear to agree closely.

* I should retain this name with some hesitation for this form, from the original description alone, which is too indefinite to enable one to be certain as to its application to a species of this genus, as now defined. But as Stimpson had Girard's original drawings for examination, and gave a more precise definition to this genus, it must be presumed that *F. Warreni*, the type, conforms to his definition.

There is a peculiar red planarian, on our coast (see pl. XL, fig. 9), which agrees well with Girard's description, so far as it goes, but my specimens had no distinct ocelli This is the species referred to *F. Warreni* by me in the Report on Invert. of Vineyard Sound, p. 633 [339]. 1873. Additional studies of fresh specimens are essential, in order to identify it.

The synonymy of the European species is still doubtful, for some European authors refer the *Planaria littoralis* Müller to this genus, while others consider it identical with *Procerodes ulvæ* Œrsted sp. As Müller's species is doubtful, I have preferred to adopt a later name, which appears to be applicable to our American form.

## Procerodes Girard.

*Procerodes* Girard, Proc. Boston Soc. Nat. Hist., vol. iii, p. 251, 1850; Stimpson, Prodromus, p. [5] 23, 1857; Diesing, Rev. Turbell., Dendrocœlen, pp. 491, 520, 1861; Jensen, op. cit., p. 74.

Size small. Body convex, thickened, more or less oblong. Head obtuse, somewhat wider than the neck. Tentacles two, near to, but distinct from, the antero-lateral margins of the head. Eyes two, well developed, situated just behind the tentacles, furnished with a transparent front lens, directed antero-laterally, and a reniform, pigmented retinal portion. Pharynx long, cylindrical. Mouth submedian, or in advance of the middle of the body.

Owing to the dark color and opacity of my specimens, when living, I was unable to observe the structure of the reproductive organs, nor did I ascertain positively whether the intestinal branches were chiefly simple or branched, but they appeared to be forked, or bilobed, in many cases, at least. My specimens are all mounted in balsam, so that sections cannot well be made, at present. The mounted specimens are very dark.

## Procerodes ulvæ (Œrsted) Stimp.

*Planaria ulvæ* Œrsted, Kroyer's Naturh. Tidssk., iv, p. 550, 1844; Diesing, Syst., vol. i, p. 205, 1850.

*Procerodes Wheatlandii* Girard, Proc. Boston Soc. Nat. Hist., iii, p. 251, 1851; Stimpson, Prod., p. 6, 1857; Diesing, Revis. Turbell., Abtheil., Dendroc., p. 520, 1861; Verrill, Invert. Vineyard Sd., etc., p. 633 [339], 1873.

*Procerodes ulvæ* Stimpson, Prod., p. 6, 1857; Diesing, Revis. Turbell., p. 521; Jensen, Turbell. Lit. Norvegiæ, p. 74, 1878.

*Planaria frequens* Leidy, Marine Invert. Fauna of Rhode I. and New Jersey, p. 11, 1855, in Journ. Philad. Acad. Nat. Sci., ser. 2, vol. iii, p. 14.

*Procerodes frequens* Stimp., op. cit., p. 6; Diesing, Rev. Turbell., p. 521; Verrill, op. cit., p. 325 [31].

PLATE XLI, FIGURE 10; PLATE XLII, FIGURES 11, 11*a*.

Body, in ordinary extension, oblong-oval, rather wider posteriorly than anteriorly, broadly rounded at the posterior end, convex dorsally and flattened beneath; the neck narrows decidedly in normal extension. Head distinctly wider than the neck, rounded in front.

Tentacles whitish, when extended a little elongated and tapered to a point, but capable of complete retraction and usually not visible in preserved specimens, though their position may be indicated by small white spots; they are situated near the antero-lateral margins of the head, but not on the margins. Eyes rather large, black, but not easily seen, owing to the dark color of the integument.* Mouth submedian.

Pharynx, as retracted in mounted specimens, long, rather narrow, cylindrical, equal to a third, or even nearly half the length of the body, its posterior end reaching back to about the posterior fourth of the body.

Color, above, dark smoky brown or blackish, often with a more or less distinct stripe of light gray or yellowish white along each side of the back, sometimes mottled with darker and lighter brown; beneath paler; tentacles and margins of head whitish.

Length 4 to $6^{mm}$; breadth 1 to $1{\cdot}5^{mm}$.

New Haven to Bay of Fundy. Point Judith, R. I., (Leidy); Manchester, Mass., (Girard). I have collected it at New Haven, Conn., Newport, R. I., Wood's Holl, Mass., Casco Bay, Me., etc. It is found also on the northern coasts of Europe. Found near low-water mark under stones, and in tide-pools, among algæ.

This species is active in its habits. It has a singular resemblance to some of the small land slugs in form and mode of progression. Its egg-capsules are unknown to me.

## Part II.—*Acœla.*

The acœlous planarians of our coast have, hitherto, received but little attention. Besides the few species noticed below, I have observed several others, but not with sufficient care to enable me to give satisfactory diagnoses. Some of the species are very common among algæ and eel-grass in shallow harbors.

The Acœla are remarkable for the absence of any distinct membrane lining the digestive cavity, so that no distinction can be made between the body-cavity and digestive cavity. This cavity is lined with a loose cellular parenchyma and has an indeterminate outline; it is often very large and usually filled with large numbers of small crustaceans, diatoms, and various other organisms.

The mouth is often at, or near, the anterior end of the body, but

* The eyes are not distinctly shown in the figure (pl. XLI, fig. 10), owing to the dark shading.

it may be near the middle of the ventral side, or even behind the middle. It is often large and is sometimes simple, but more frequently it is surrounded by a slightly developed muscular ring or collar; more rarely there is a rudimentary pharynx. The integument is generally filled with clusters of rhabdites. There is often a peculiar glandular frontal organ opening at the anterior end.

The brain, longitudinal nervous trunks, and nervous branches are present, but not so well-defined as in the previous groups. A pair of ocelli may be present, but are oftener absent. A median otocyst is almost always present. Posterior tentacle-like organs or cirri are sometimes present.

The reproductive organs are variable in structure and position. Sometimes there is but a single genital pore, but usually there are two median ones, posteriorly situated, the male orifice being behind the female. The ovaries are usually lateral and paired; special yolk-glands are not developed. A bursa seminalis or spermatheca may be present, or absent; sometimes there are two. The testes are folicular and imbedded in the parenchyma. The penis may be simple or armed; a seminal vesicle is usually present. Eggs are usually enclosed in capsules.

The external form is exceedingly varied. Many species are flat, broad-elliptical or ovate; others are long and narrow; some are nearly terete; others are angular in outline. Some species (*Convoluta*) habitually curl the sides of the body inward and downward, so that they appear narrower than they really are. The size is generally small, the colors often bright.

### Family, APHANOSTOMIDÆ Graff, Monog.

Body usually more or less flattened, elliptical, ovate, or cordate, long or short, frequently with the sides curved downward. Mouth ventral, behind the otocyst, often median or submedian. Pharynx rudimentary or wanting. Otocyst present. Ocelli sometimes two, often absent. Genital orifices separate. Penis simple, unarmed. Bursa seminalis well-developed, with one, two, or several tubular outlets.

### Aphanostoma Œrsted.

Kroyer's Naturhist. Tidssk., ser. 2, vol. i, p. 417, 1845; Carus, Fauna Med., p. 139, 1884; Graff, Monog., p. 220; Graff, Turbell. Acœla, pp. 55, 59, 1891.
*Aphanostomum* Jensen, Turbell. Lit. Norvegiæ, p. 22, 1878.

Body more or less flattened, oblong, ovate, or elliptical; usually narrowed posteriorly. Mouth circular, before or near the middle.

A rudimentary pharynx. Otocyst single, near the anterior end. Otolith usually well developed. No ocelli. Frontal glandular organs present.

Genital apertures, posterior; the male behind the female. Spermarian folicles numerous, lateral. Openings of the *bursa seminalis* without chitinous parts. Ovaries paired, lateral.

## Aphanostoma diversicolor Œrsted.

Kroyer's Naturhist. Tidssk., ser. 2, vol. i, p. 417, 1845; Carus, op. cit., p. 139; Graff, Monogr., p. 220; Graff, Turbell. Acœla, p. 59, pl. 5, fig. 4, 1891, section.

*Aphanostomum diversicolor* Jensen, Turbell. ad Lit. Norvegiæ, p. 26, pl. I, figs. 12 to 21, 1878, anatomy.

PLATE XLII, FIGURE 8.

Body very changeable, in extension usually long-oval or elliptical, obtuse anteriorly, or tapering to both ends, but most so posteriorly; only a little depressed; in contraction short-ovate and thick.

Color variable, generally with a sulphur-yellow spot on the anterior end and another on the back; a large spot of bluish or violaceous usually covers more or less of the back, and is sometimes divided posteriorly by a V-shaped white line; lateral borders whitish. Mouth subcentral beneath. A cluster of frontal glands is connected with a pore at the anterior end of the body. Otocyst small, much obscured by the pigment, gibbous, situated at about the anterior eighth.

Length, in extension, 1·5 to $2^{mm}$; breadth 0·25 to $0·35^{mm}$.

Newport, R. I., July 29, 1880, among algæ at low-water mark. It occurs on the coasts of Norway and Great Britain, and in the Mediterranean at Naples, Trieste, etc.

## Aphanostoma aurantiacum sp. nov.

PLATE XLII, FIGURES 10, 10*a*.

Body long-ovate in extension, depressed, broadest in front of the middle, usually bluntly rounded anteriorly, but very soft and changeable. Otocyst rather large, conspicuous, situated at about the anterior eighth, gibbous, containing an inverted cup-shaped or bell-shaped otolith, situated at the anterior side of the nearly circular and transparent vesicle. The otolith is almost continually in motion, while living. Ground-color light ocher-yellow, everywhere thickly covered with small, distinct spots or specks of bright orange-red, thus giving the whole surface an orange-yellow color, paler towards the margins. Reproductive organs are unknown.

Length about $1{\cdot}5^{mm}$; breadth $0{\cdot}35$ to $0{\cdot}50^{mm}$.

Newport, R. I., at low-water mark, among algæ, July 29, 1880.

This species is only provisionally referred to the genus *Aphanostoma,* for its internal anatomy was not studied. The specimens were lost.

**Aphanostoma olivaceum** sp. nov.

PLATE XLII, FIGURE 9.

Body ovate, broadest anteriorly, rather thick, convex. Otocyst conspicuous, at about the anterior eighth. Mouth rather behind the middle; digestive cavity large, behind the middle; in the example figure it contained an amphipod crustacean.

The ovaries are large, lateral; posteriorly, the oviducts are filled with two or three rows of large ova, which are dark green in color. The female orifice is about midway between the posterior border of the digestive cavity and the male orifice. The latter is near the posterior margin, in the center of a pale spot. It is flanked on each side by two small vesicles (probably seminal vesicles); two larger saccular organs (perhaps the posterior part of *vasa deferentia*) run forward and diverge, but their nature was not certainly ascertained owing to the dark color of the integument. The form and structure of the penis and spermatheca were not observed, for the same reason

Color dark olive-green, or brownish green.

Length $2^{mm}$; breadth about $1{\cdot}5^{mm}$.

Provincetown, Mass., at low-water mark, among filamentous algæ, Aug. 14, 1879.

This species is referred to *Aphanostoma* only provisionally, for its reproductive organs are imperfectly known. It has considerable resemblance to certain species of *Mecynostoma.*

**Polychœrus** Mark.

Festschrift für Leuckart, p. 298, Oct., 1892.

Body flattened, changeable in outline, usually cordate or ovate, narrowest anteriorly, emarginate or bilobed posteriorly, with one to five slender, contractile caudal cirri. The lateral margins are usually not inflexed. Mouth central or nearly so, with simple muscular margins. No proper pharynx. Digestive cavity large, separated posteriorly from the genital area by tissues more dense than those of other parts.

Otocyst small, round, sometimes indistinct or wanting; otolith cup-shaped.

Brain distinctly bilobed; each ganglion is somewhat stellate in form, and gives off three main nerves: anterior, lateral, and poste-

rior. The superficial nerves are extensively anastomosed, so as to form a network of irregular meshes.

Ovaries large, on each side of the digestive cavity; oviducts large, often much distended posteriorly by clusters of large mature ova. Spermarian folicles numerous, situated on each side of, and often extending nearly or quite around, the digestive cavity. *Vasa deferentia* large, more or less saccular posteriorly, when distended with their contents, and connected with a circular seminal vesicle surrounding the base of the penis.

The penis is unarmed and in contraction ovoid or conical, but rather long, tapered, and usually somewhat curved when fully extended. (See plate XLIV, figs. 6, 8, 9).

The male orifice is near the posterior margin, in front of the median caudal cirrus.

The female orifice is situated a little farther forward and communicates with a simple tubular vagina, which runs up dorsally beneath the saccular spermatheca.

Spermatheca or *bursa seminalis* is large, complex, reniform or cordate, with numerous chitinous outlet-tubes scattered over the surface.

Rhabdites and pigment-corpuscles are contained in the integument.

### Polychœrus caudatus Mark.

Mark, op. cit., pp. 298–309, pl. XXI, figs. 1–22, anatomy.

PLATE XLI, FIGURES 11, 11*a*; PLATE XLIV, FIGURES 6 TO 10.

Body depressed, flat or concave beneath, somewhat convex above, and often gibbous or swollen dorsally, when filled with ova; in extension varying from broad cordate to narrow-ovate, according to the state of contraction; front end bluntly pointed or evenly rounded; sides often nearly parallel; posterior end usually deeply emarginate, with a broad median notch and rounded lobes each side of it; margin thin, sometimes inflexed, especially when swimming.

Caudal cirri one to five. In adult examples there are generally three slender, pale, translucent caudal cirri, one of which is median, while the others, which are usually a little smaller, arise from near the inner border of the posterior lobes. Sometimes, in large specimens, another smaller pair is developed external to the latter, on the posterior lobes (pl. XLIV, fig. 6). In young specimens the median cirrus appears first. The cirri all arise at a slight distance from the margin, on the dorsal side. Each one can be retracted into a small, basal, bulb-like, muscular, invaginated cavity of the

integument. The caudal cirri are often lost by injuries, but are quickly reproduced.

The otocyst is small, circular, situated well forward at about the anterior fourth; otolith cup-shaped. In some specimens the otocyst appears to be rudimentary or wanting; in many it can scarcely be seen while living, owing to its small size and the great amount of pigment in the integument; in others it is easily visible.

Mouth central or subcentral, simple, circular or transversely elliptical, highly dilatable, surrounded by circular muscular fibers. The digestive cavity is capacious, more or less irregular in outline. It is usually filled with various Entomostraca and other small crustaceans.

The genital openings are small, round, near together, and usually not very distinct. The male orifice is a little in advance of the base of the median caudal cirrus. The penis, as seen from beneath and foreshortened in mounted specimens, is usually ovoid or conical; when protruded it is rather elongated, cylindrical or tapered, often curved, with a conical tip. Its base is surrounded by a circular seminal vesicle (pl. XLIV, figs. 6–10, *r*) which receives the *vasa deferentia* (*d*) on the right and left sides. The latter are voluminous, more or less saccular and contorted posteriorly, when filled with their contents.

The spermarian folicles (*t*) are small, numerous, pyriform, and extend along each side above and partly external to the ovaries and oviducts, as far as the anterior margin of the digestive cavity, where they converge and nearly or quite blend at the median line, when fully developed.

The oviducts are large and saccular and lie close to the digestive cavity, on each side; posteriorly they are often much swollen by large clusters of relatively large mature ova, of which there are often six to ten, arranged in two or three rows, in each cluster; those farthest back often crowd against and more or less distort and conceal the spermatheca and other organs, and make that part of the body gibbous externally.

The spermatheca (figs. 6, 7, 8, *s*) is large, glandular and saccular dorsally, variable in shape, but when least crowded it is generally broad-cordate or reniform; the emargination of the posterior side includes the female orifice and vagina. Its ventral surface is covered with small brownish, chitinous, conical outlet-tubes or "mouth-pieces," differing in size and varying in number in different specimens from six or eight up to thirty or forty; most frequently twelve to fifteen fully developed ones and several smaller

ones can be seen. Each of these is surrounded at base by a circle of glandular cells.*

In small specimens the male organs are often well developed before the female organs appear.

According to Professor Mark, the spermatozoa are long and filiform, thickest in advance of the middle, with a very slender anterior portion, more active and more attenuated than the posterior portion.

Color usually brick-red or dark orange-red, sometimes pale red, with a central paler spot over the digestive cavity, and usually with a circular or horse-shoe-shaped region of darker brownish red color over the ovaries, and almost entirely surrounding the digestive region, interrupted posteriorly; margins pale; caudal cirri translucent, whitish; ventral surface yellowish.

The orange color, according to Professor Mark's observations, is due to clusters of two kinds of minute pigment-corpuscles. Of these, the most numerous are greenish yellow; the others, which are smaller and less numerous, are purplish. The pale median patch is caused by whitish, mostly rod-like, corpuscles. Clusters of cigar-shaped rhabdites are also scattered in the integument.

Length 3 to $4^{mm}$; breadth 1·5 to $2^{mm}$.

Common from Great Egg Harbor, N. J. to Casco Bay, Me.; especially in sheltered harbors, adhering to eel-grass (*Zostera*) and creeping over the vegetable debris, shells, etc., on the bottom in shallow water, where it is often extremely abundant.

I have taken it in large numbers, especially in New Haven Harbor, 1865 to 1870; Noank, Conn., 1874; Newport, R. I., 1880; Wood's Holl, Mass., 1871, 1875, 1881 to 1887. At Quahog Bay, Me., in 1873, I found it in small numbers, but have not observed it farther north.

I have been familiar with this species for many years, and have had several drawings and descriptions of it made as early as 1874 and 1875, but had put them aside with those of various other Turbellaria.† Not knowing that Professor Mark had worked upon

* Professor Mark (op. cit.) describes some of these "mouth-pieces," observed by him, as filled with the filiform spermatozoa, with clusters of them hanging from the basal portion. He also observed large masses of spermatozoa in the dorsal cavities of the spermatheca, with which the "mouth-pieces" communicate. The vagina terminates in proximity to the dorsal cavities of the spermatheca.

† In the summer of 1874, while in charge of the invertebrate zoölogy of the U. S. Fish Com., at Noank, Conn., I had the pleasure of studying this species in company with the late Professor Joseph Leidy, who was much interested in its anatomy. For that reason in my MSS. notes I had named it in honor of Professor Leidy.

it, and before his excellent paper had been published, I had resumed the study of the species and prepared descriptions of the genus and species for this article. Having received Professor Mark's paper, just as the last pages of my own were going to press, I have been able to add several additional anatomical facts of importance, which I had not personally observed. Professor Mark, in his paper, moreover, describes in detail the nervous system, and also numerous additional features not here referred to, or only briefly mentioned.

My own conclusions, in regard to the essential structure and affinities of the genus, were perfectly in accord with those of Professor Mark. The few additional details that I have given in the preceding description are due to the larger series that I have had for study, or to their different modes of preservation.

Professor Mark informs me that he has found the egg-capsules of this species in abundance on dead shells and stones in the harbor of Wood's Holl, Mass. They are circular, flat on the lower side, by which they are attached, and a little convex on the upper side, with thin margins. I have not met with them myself. In several instances I have seen living young individuals in the interior of the body of adults, but it is quite possible that they had been swallowed as food with other small prey. The mode of depositing the egg-capsules is unknown. There seems to be no special opening adapted to that purpose. Possibly they escape from the mouth. The specimen figured on pl. XLI, fig. 11*a*, appears to contain egg-capsules in process of formation, but as the specimen was not preserved, this cannot now be confirmed.

*Doubtful Species.*

**Typhlocolax acutus** Stimpson, Prod, p. 3, 1857.

*Typhlolepta acuta* Girard, in Stimpson, Invert. Grand Manan, p. 27, 1853; Diesing. Rev. Turbell., p. 523, 1861.

"Body depressed, ovoid, elongated, posteriorly rounded; anterior extremity terminating in an acute point; mouth underneath, and situated at about the middle of the body. Length about a sixteenth of an inch. Ground color pale, with reddish confluent blotches above. Found in considerable numbers creeping over the surface of *Chirodota lævis*."

Dr. Stimpson (Prodromus, p. 3) referred this genus and species to the Digonopora, but its internal structure was not known to him. It is more probable that it belongs to the Acœla or Rhabdocœla. I have not observed it myself, although I have collected numerous specimens of the holothurian, on which it is said to be parasitic, in the original locality.

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# EXPLANATION OF PLATES.

## PLATE XL.

Figure 1.—*Imogine oculifera* Girard, p. 475. Dorsal view of a young living specimen with dendritic, rose-colored makings; × 20. Wood's Holl, Mass.

Figure 2.—*Eustylochus ellipticus* (Girard) V., p. 467. Dorsal view of a living specimen, much compressed; × 6. Savin Rock, near New Haven, Conn., 1868.

Figure 3.—*Stylochus zebra* V., p. 463. Dorsal view of a living specimen; × 4. Wood's Holl, Mass., Sept. 23, 1882.

Figure 4.—*Planocera nebulosa* Girard, p. 472. Dorsal view of a living specimen, × 5. New Haven, Conn., 1870.

Figure 5.—*Leptoplana ellipsoides* Girard, p. 483. Dorsal view of a living specimen; × 5. Eastport, Me., 1870, at low-water. Color orange-brown with irregular spots of dark brown and small specks of white. (The clusters of ocelli occupy unusual positions, owing to a peculiar state of contraction).

Figure 6.—*Leptoplana ellipsoides.* Dorsal view of a large example, from life, in the act of creeping; × 4. Eastport, Me., August 10, 1872.

Figure 7.—*Discocelis mutabilis* V. (?), p. 493. Dorsal view of a young living specimen; much enlarged. Surface, Wood's Holl, Mass., August 16, 1882. Transparent, with yellow spots around the margin.

Figure 8.—*Leptoplana angusta* V., p. 485. Dorsal view of a young living example, × 10. Provincetown, Mass., from the bottom of a whaler.

Figure 9.—*Fovia affinis* (?). See foot-note, p. 505. Dorsal view; × 6. Noank, Conn., in harbor, July 16, 1874.

Figure 10.—*Bdelloura candida* Girard, p. 499. Ventral view (*a*), from life; dorsal view (*b*); about natural size; 10*b*, the same, posterior sucker; × 4.

Figures 1, 3, 6, 7, 10 were drawn from life by J. H. Emerton; figure 9 by J. H. Blake; the rest by the author.

## PLATE XLI.

Figure 1.—*Eustylochus ellipticus* (Girard) V., p. 467. Dorsal view of a living specimen; × 4. 1*a*, the same, one of the tentacles; more enlarged. Newport, R. I., on piles of railroad bridge. August 20, 1880.

Figure 2.—*Eurylepta maculosa* V., p. 495. Dorsal view of a living specimen; × 5. Wood's Holl, Mass., on piles, July 15, 1881.

Figure 3.—The same. Dorsal view of a living specimen; × 5. Naushon Island, at low-water mark, August 20, 1887.

Figure 4.—*Trigonoporus dendriticus* V., p. 491. Dorsal view of a living specimen; × 4. Sta. 317, off Cape Cod, 25 fath., 1879. Color light pink with a pale central area. (The clusters of ocelli are too angular and definite.)

Figure 5.—*Trigonoporus folium* V., p. 487. Dorsal view from life; × 2. Fig. 5*a* the same, ventral view. Sta. 784, off Point Judith, R. I., in 20 fath., 1880.

Figure 6.—*Trigonoporus folium* V., p. 487. Dorsal view of a living specimen; ×5. Sta. 134, off Cape Ann, Mass., in 26 fath., July 23, 1878. Color yellowish brown with the gastric region and margins pale; ganglions and nerves pink.

Figure 7.—*Leptoplana variabilis* (Girard). No. 20, p. 480. Dorsal view, from life; ×5. Sta. 156, off Gloucester, Mass., 42 fath., Aug. 15, 1878. Color yellowish brown with pale margins and central stripe. The clusters of cerebral ocelli are too long and too divergent.

Figure 8.—*Bdelloura candida* Girard, p. 499. Dorsal view of a living specimen slightly compressed between glasses, and seen by transmitted light; ×3.

Figure 9.—*Fovia affinis* (Œrs.) Stimp. (var. *grisea* V.), p. 504. Dorsal view of a living specimen; ×6. Fig. 9*a*, the same specimen strongly contracted while living; ×6. Fig. 9*b*, the same; dark-brown variety. Ventral view of a living specimen, seen by transmitted light; ×3. Gloucester, Mass., on eel-grass (*Zostera*), 1878.

Figure 10.—*Procerodes ulvæ* (Œrs.) Stimp., p. 506. Dorsal view of a living specimen; ×8. Casco Bay, at low-water mark, 1873. The ocelli are not shown, owing to the dark shading.

Figure 11.—*Polychærus caudatus* Mark, p. 511. Dorsal view of a living specimen; ×20. Noank, Conn., July 30, 1874. The caudal cirri should have been left without color.

Figure 11*a*.—The same. Another individual seen as a transparent object, slightly compressed between glasses. Wood's Holl, Mass., 1881. The bilobed body, covering the region of the spermatheca, was probably an egg-capsule (or two of them) in process of formation (see p. 514).

Figures 3 and 11 were drawn by J. H. Blake; 4, 6, 7, 8, 9*b* by the author; the rest by J. H. Emerton.

PLATE XLII.

Figure 1.—*Eustylochus ellipticus* (Girard) V., p. 466. Dorsal view of a living specimen; ×6. New Haven Harbor, Oct., 1892.

Figure 1*a*.—The same specimen. Ventral view; ×6; *b*, one of the tentacles; *c*, brain: *f*, pharynx; *m*, mouth; *d*, vas deferens; *r*, seminal vesicle; *p*, penis; *p'*, penis-bulb and granular gland; ♂, male orifice; ♀, female orifice; *s*, spermatheca; *s'*, duct of the same; *v*, vagina: *u*, large oviduct or uterine sac; *z*, orifice of the median nephridial tube (*z'*).

Figure 1*b*.—The same. Male genital organs of a young mounted specimen; much enlarged; *d*, *d*, vasa deferentia; *r*, seminal vesicle; *p*, penis; *p'*, penis-bulb and granular gland. New Haven Harbor, Oct., 1892.

Figure 2.—*Stylochus zebra* V., p. 463. Ventral view of a living specimen; ×4; *f*, pharynx; *m*, mouth; *d*, vas deferens; ♂, male orifice; ♀. female orifice; *w*, shell-glands. Off New Haven.

Figure 2*a*.—The same. Dorsal view of the head and anterior portion of another specimen from the same locality, from life; ×4.

Figure 3.—*Planocera nebulosa* Girard, p. 473. Genital organs of a young specimen; much enlarged. New Haven Harbor, Oct., 1892. *d*, vas deferens; *r*, seminal vesicle; *r'*, its duct; *p*, penis and penis-sheath; *p'*, penis-bulb and granular gland; ♂, male orifice; ♀, female orifice; *v*, vagina; *s*, spermatheca.

Figure 4.—*Trigonoporus dendriticus* V., p. 491. Ventral view of a specimen mounted in balsam; ×6. Station 317, off Cape Cod, 1879.

Figure 4*a*.—The same specimen. Brain and clusters of ocelli; × 25; *c*, brain; *e*, cerebral ocelli; *e'*, dorsal ocelli; *g*, *g*, anterior branches of the stomach. Fig. 4*b*, cerebral ocelli of the same specimen; × 25.

Figure 5.—*Trigonoporus folium* V., p. 487. Ventral view of a specimen mounted in balsam; × 6. Eastport, Me., 1870. Fig. 5*a*, ocelli of the left side of the same specimen, × 20; *e*, cerebral cluster; *e'*, dorsal cluster. Fig. 5*b*, ocelli of the right side of the same specimen; × 25; *e*, cerebral cluster; *e'* dorsal cluster.

Figure 6.—*Discocelis mutabilis* V., p. 493. Dorsal view of the original, type-specimen mounted in balsam; × 8. Thimble Islands, 1872.

Figure 6*a*.—The same specimen. Clusters of ocelli; × 25; *e*, cerebral, and *e'*, dorsal clusters of ocelli.

Figure 7.—*Discocelis mutabilis* V. (?), young, p. 494. Dorsal and cerebral ocelli of a mounted specimen; × 25. Wood's Holl, Mass., 1882.

Figure 8.—*Aphanostoma diversicolor* (Œrst.), p. 509. Dorsal view of a living specimen; × 20. Newport, R. I., 1880.

Figure 9.—*Aphanostoma olivaceum* V., p. 510. Ventral view of a living specimen; × 12; *ot*, otocyst; *g*, digestive cavity containing an amphipod crustacean; *s*, large oviduct containing ripe ova; ♀, female orifice, ♂, male orifice and penis.

Figure 10.—*Aphanostoma aurantiacum* V., p. 509. Dorsal view of a living specimen; × 20.

Figure 10*a*.—The same specimen. Otocyst; more enlarged.

Figure 11.—*Procerodes ulvæ* (Œrst.), p. 506. Dorsal view of head of a living specimen; × 12. Casco Bay, Me., 1873.

Figure 11*a*.—The same. Dorsal view of another specimen mounted in balsam; × 8; *o*, ovaries; *f*, pharynx; *p*, penis; ♀ ♂, common genital orifice. (In this figure the number and form of the gastric branches are partly diagramatic, for many of them could be seen only indistinctly).

Figures 1, 1*a*, 2, 2*a*, 4, 4*a*, 5, 6 were drawn from nature by A. H. Verrill; 8 and 10 were drawn from life by J. H. Emerton; the rest are camera-lucida drawings by the author.

PLATE XLIII.

Figure 1.—*Leptoplana virilis* V., p. 478. Ventral view of the type-specimen, stained and mounted in balsam; × 6. Sta. 307, Off Cape Cod, Mass., 31 fath, 1879.

Figure 1*a*.—The same specimen. Posterior portion, ventral view; × 15; *f*, pharynx; *r*, seminal vesicle; *k*, granular gland and penis-bulb; *p*, penis; *q*, penis-sheath; *t*, *t*, spermarian folicles or testes: ♂, male orifice; ♀, female orifice; *o*, *o*, *o*, ovarian folicles; *v*, ventral portion of vagina; *v'*, dorsal portion of the same; *s*, spermatheca; *s'*, anterior portion of the same and its connection with the vagina; *w*, shell-glands; *u*, one of the uterine sacs.

Figure 2.—*Leptoplana variabilis* (Girard) V., young, p. 480. Dorsal view of a mounted specimen; × 8. Gloucester, Mass., in tide-pool, 1878.

Figure 3.—*Leptoplana variabilis* (Girard) V. No. 20. Ventral view of a mounted specimen; × 6. Sta. 156, off Cape Ann, Mass., 42 fath., 1878.

Figure 3*a*.—The same specimen. Genital organs, dorsal view; × 15; *d*, *d*, vasa deferentia; *k*, granular gland; *p*, penis, *q*, penis-sheath; ♀, female orifice; *v*, ventral portion of vagina; *v'*, dorsal portion of the same; *s*, spermatheca; *s'*, anterior division of the same and its connection with the vagina; *w*, *w*, shell-glands; *u*, *u*, large oviducts or uterine sacs; *u'*, duct of the same leading to the vagina; *x*, orifice of the nephridial duct (?); *g'*, *g'*, posterior gastric branches.

Figure 3*b*.—The same specimen. Ocelli and brain; × 25; *c*, brain; *e*, cerebral ocelli; *e'*, dorsal ocelli.

Figure 4.—*Leptoplana ellipsoides* Girard, p. 483. Ocelli; × 25; *e*, cerebral, and *e'*, dorsal clusters. Eastport, Me., 1872.

Figure 4*a*.—The same. Dorsal view of the genital organs; × 15. The lettering is the same as that of 3*a*. Figure 4*b*.—The same parts; ventral view.

Figure 5.—*Trigonoporus dendriticus* V., p. 491. Type-specimen. Sta. 317. Ventral view of the genital organs; × 25; ♂, male orifice; *p*, penis; *p'*, penis-bulb; *q*, penis-sheath; *k*, granular gland; ♀, anterior female orifice; ♀', posterior female orifice; *w*, shell-glands.

Figure 6.—*Polychærus caudatus* Mark, p. 512. Ventral view of a mounted specimen; × 15. Wood's Holl, Mass., 1881. This specimen is noteworthy for having five caudal cirri. The digestive cavity contains numerous small crustaceans.

Figure 7.—The same. Ventral view of a mounted specimen having three caudal cirri; × 15; ♀, female orifice; *s*, spermatheca; *o*, one of the ovaries; *t*, spermarian folicles or testes; *p*, retracted penis and male orifice.

Figure 8.—The same. Ventral view of the genital organs of a mature specimen; × 30; *d*, *d*, vasa deferentia; *r*, seminal vesicle; *p*, penis exserted; ♀, female orifice; *s*, spermatheca or bursa seminalis; *o*, cluster of mature eggs.

Figure 9.—The same. Male genital organs of another specimen with the penis (*p*) fully exserted; *r*, seminal vesicle; × 30.

Figure 10.—The same, another specimen. A partially profile view of the genital organs; × 30; ♀, female orifice; *v*, vagina; *p*, penis partly protruded; *d*, *d*, vasa deferentia; *r*, seminal vesicle.

Figure 11.—*Bdelloura candida* Girard, p. 499. Ventral view of a specimen containing a nearly mature egg-capsule; × 6; *c*, brain and ocelli; *h*, longitudinal muscles of the sucker; *n*, one of the longitudinal nerve-trunks; *n'*, frontal nerves; *g*, anterior division of the stomach; *f*, pharynx; *o*, *o*, ovaries; *ca*, egg-capsules; *u*, uterine sac or accessory gland.

The large chitinous egg-capsule occupies the entire thickness of the body, no organs appearing over its central part, on either side, except the thin, stretched integument; its front edge lies above the end of the pharynx, and the *vasa deferentia* run above its lateral borders. How it is expelled is not known.

Figures 1, 1*a*, 2, 3, 3*a*, and 11 were drawn from nature by A. H. Verrill; the rest by the author.

PLATE XLIV.

Figure 1.—*Stylochus frontalis* V., p. 465. Ocelli and tentacles of the type-specimen, mounted in balsam; × 30; *b*, *b*, tentacles retracted; *e*, *e*, cerebral clusters; *e'*, *e'*, dorsal clusters at base of tentacles; *e''*, *e''*, scattered frontal ocelli.

Figure 2.—*Leptoplana angusta* V., p. 485. Ventral view of one of the type-specimens, mounted in balsam; × 12; *c*, brain; *f*, pharynx; *m*, mouth; *t*, *t*, spermarian folicles; *d*, vas deferens; *p*, penis and penis-sheath; *v*, vagina and shell-glands; *s*, spermatheca; ♀, female orifice; *u*, uterine sac filled with mature ova.

Figure 2*a*.—The same. Brain and ocelli; × 30; *c*, brain; *e*, cerebral, and, *e'* dorsal clusters.

Figure 3.—The same. Reproductive organs of another mounted specimen; × 20; *p*, penis; *q*, penis-bulb and sheath; *v*, female orifice and ventral portion of vagina; *v'*, dorsal portion of vagina; *u*, *u*, large oviducts or uterine sacs filled with mature ova.

Figures 4, 4*a*.—*Trigonoporus folium* V., p. 487. Original type-specimen mounted in balsam. 4, ventral view of the genital organs, × 15; ♂, male orifice; *p*, penis; *k*, penis-bulb and granular gland; ♀, anterior female orifice in a cup-like depression; *v*, vagina mutilated; *w*, *w*, shell-glands. 4*a*, dorsal view of the same parts, with the same lettering, and *p'*, penis-sheath; *v'*, dorsal portion of vagina; ♀', posterior female orifice.

Figure 4*b*.—The same specimen; *e*, cerebral ocelli; *e'*, dorsal ocelli; × 30.

Figure 4*c*.—The same specimen. Part of front margin and marginal ocelli; × 30.

Figure 4*d*.—The same specimen. Mouth and retracted pharynx, ventral view; × 10.

Figure 5*b*.—The same. Brain and ocelli of a specimen from sta. 134, 1878. Ventral view; × 50; *c*, *c*, cerebral ganglions; *e*, cerebral ocelli; *e'* dorsal ocelli; *n*, *n*, large frontal nerves; *g*, median anterior gastric branch.

Figure 6.—The same. Genital organs of a specimen from sta. 301. Ventral view; × 30; ♂, male orifice; *p*, penis; *p'*, interior of penis-bulb; *q*, penis-sheath; *k*, granular gland; ♀, anterior female orifice; ♀', posterior female orifice; *v*, ventral part of vagina; *v'*, dorsal part of vagina; *w*, shell-glands; *u*, supposed orifice of nephridial duct.

Figure 7.—The same. Genital organs of a specimen from sta. 182, 1878. Ventral view; × 30. Lettering is the same as in fig. 6.

Figure 8.—*Bdelloura candida* Girard, p. 499. A specimen stained with borax-carmine and picric acid and mounted in balsam. New Haven. Oct., 1892. Ventral view; × 10; A, posterior sucker; *c*, *c*, two cerebral ganglions of the brain, and the ocelli; *c'*, main frontal nerves (five pairs are shown, by stippled lines, with a few of their branches); *n*, one of the great longitudinal nerve-trunks; *n'*, posterior commissure uniting the nerve-trunks, from this part large numbers of branches diverge to the borders of the posterior sucker; *nn*, one of the several serial transverse commissures uniting the nerve-trunks; *n"*, marginal nerve and exterior branchlets; *f*, pharynx, retracted; *f'*, its open end; *g*, anterior median division of the stomach; *g'*, one of the two main posterior divisions of the stomach; *g"*, one of the transverse lateral gastric branches. (The stomach and its branches are left unshaded for greater clearness; they are usually more deeply colored than the other organs); *o*, left ovary; *o'*, its duct: *y*, vitellaria or yolk-glands: *x*, left accessory female gland or "uterine sac" and its convoluted duct, going to the female genital organs; *p*, penis and penis-sheath; ♀ ♂, common genital orifice; *t*, *t*, testes or spermarian folicles.

Figure 8*a*.—The same. Ventral view of the posterior part of a living specimen compressed between glasses; × 20. The gastric branches are distended by the pressure and the nervous system is omitted. The lettering is the same as in fig. 8. with the following additions; *d*, vas deferens of the right side; *r*, seminal vesicle; *v*, vagina.

Figure 8*b*.—The same. Side and profile views of an egg-capsule that was attached by both ends; × 8.

Figures 2, 3, 4, 4*a*, 8, 8*a*, 8*b* were drawn from nature by A. H. Verrill; the rest by the author.

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## ERRATA.

Page 166, fifth line from bottom, for melancholia, read melancholica.

Page 170, eleventh line from bottom, for C. crocata, read G. crocata.

Page 170, seventh line from bottom, for C. bivittata, read G. bivittata; also, p. 171, sixth and sixteenth lines from top and ninth line from bottom.

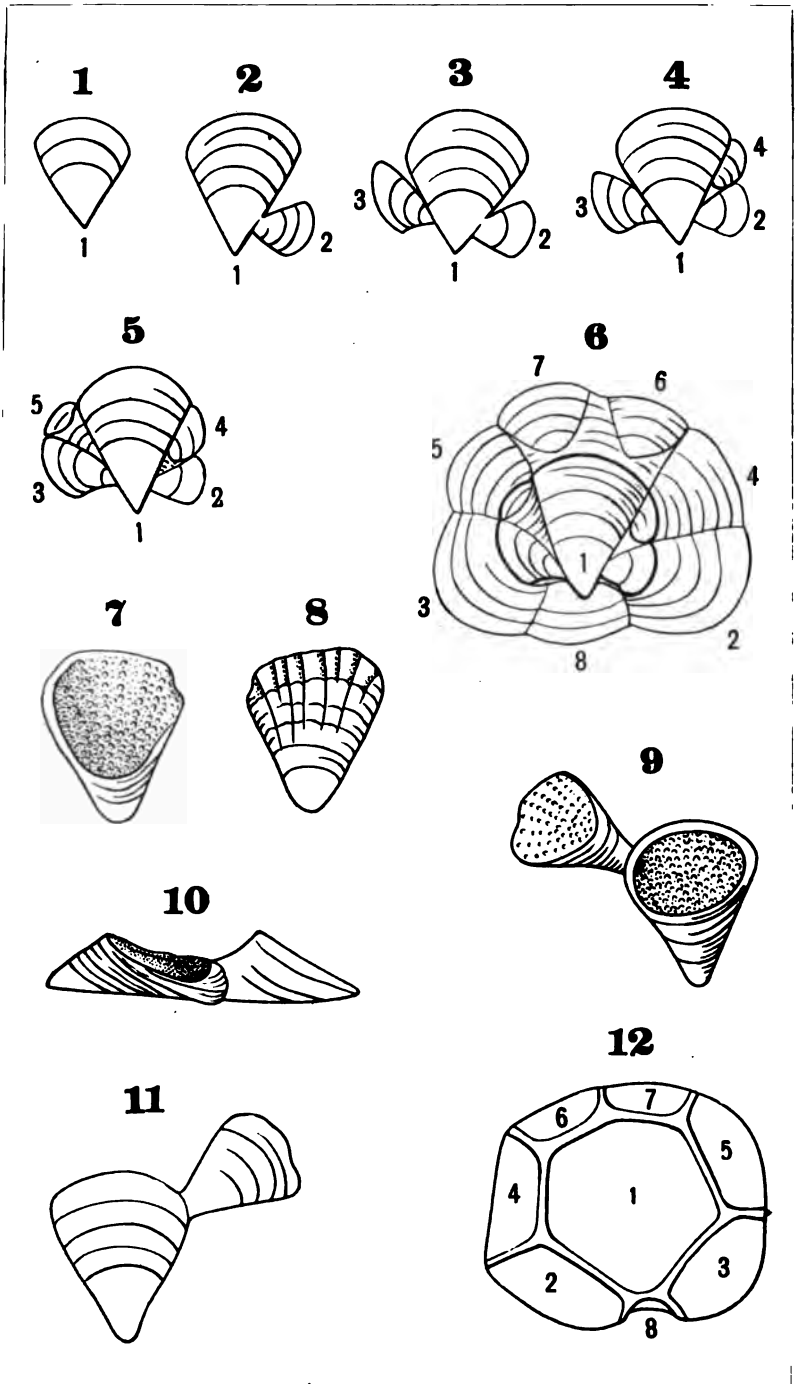
Page 191, ninth line from top, for Cælotes, read Cœlotes.

Page 233, fourth line from bottom, for cius, read Icius.

Page 317, sixth line from top, for A. flammea nigrescens, read S. flammea nigrescens.

Page 337, fourteenth line from bottom, for schlateri, read sclateri.

Page 442, second line from top, for Schizonertina, read Schizonemertina.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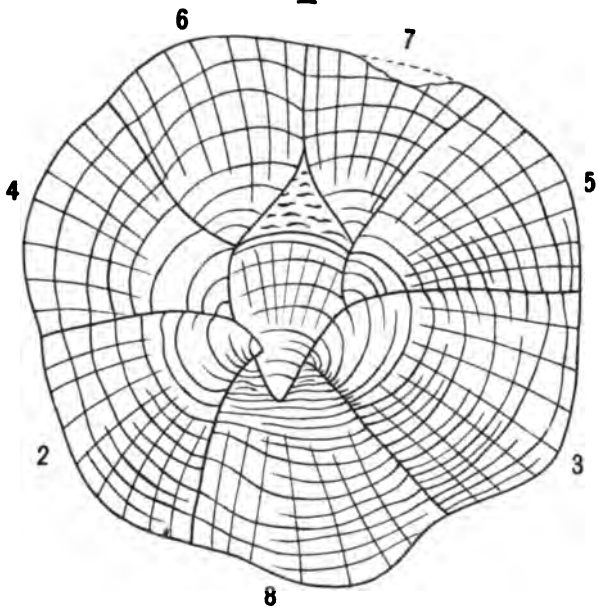


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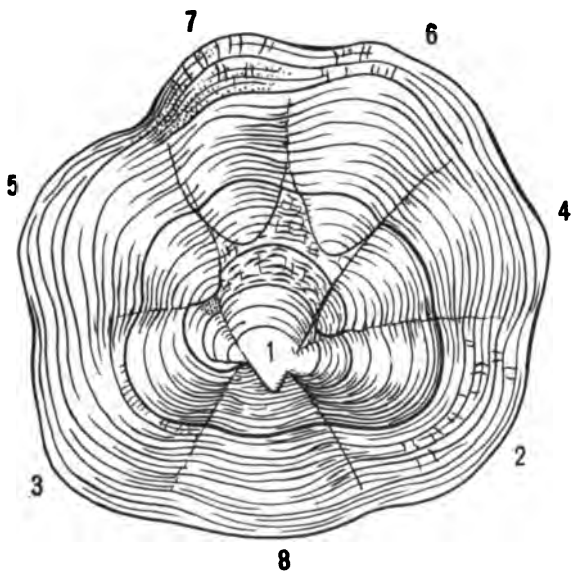

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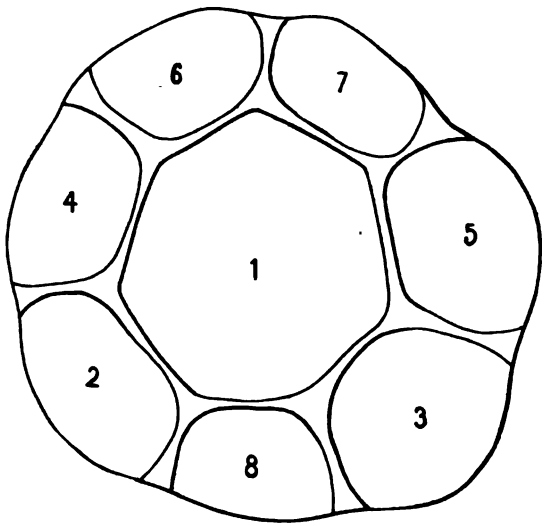


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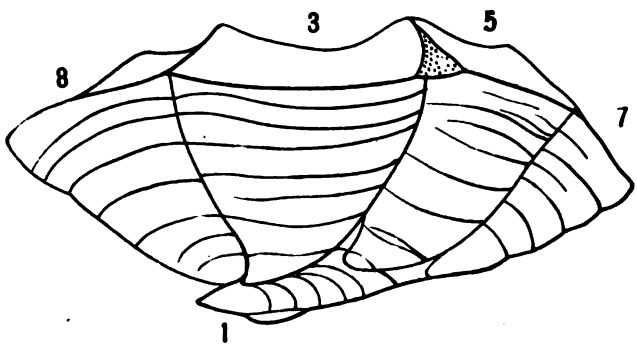

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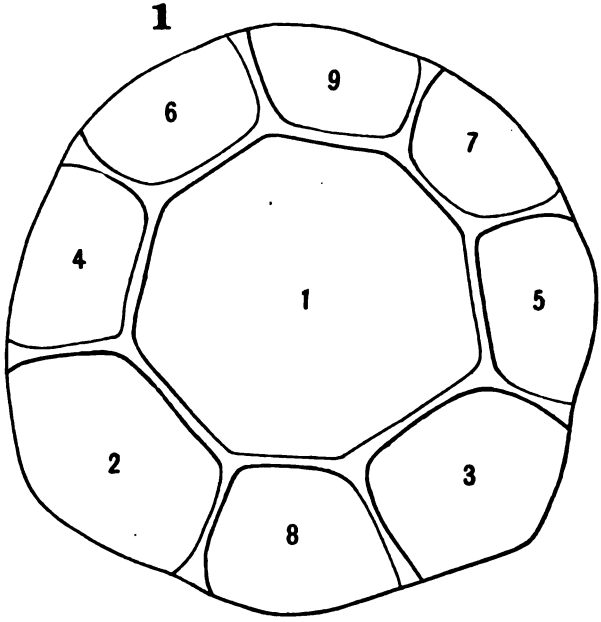


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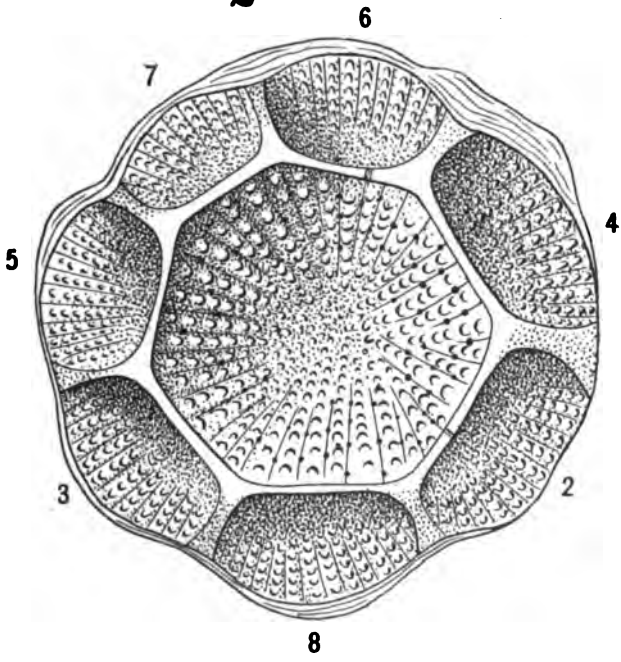

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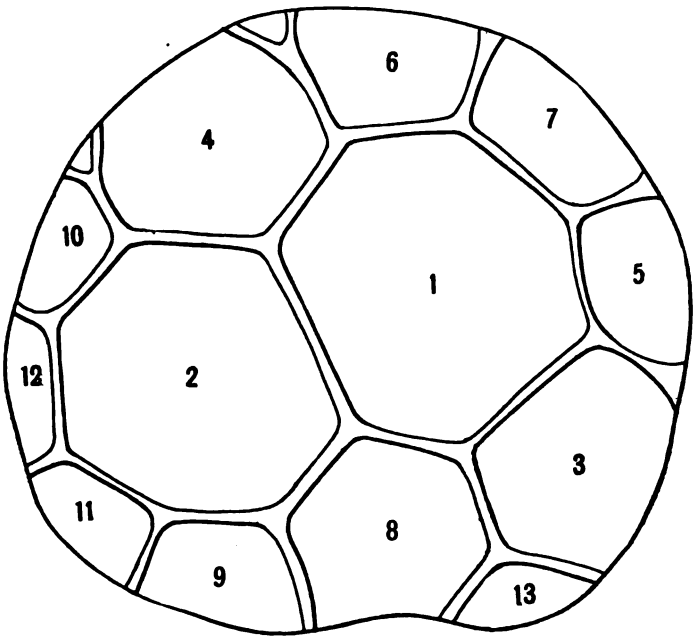


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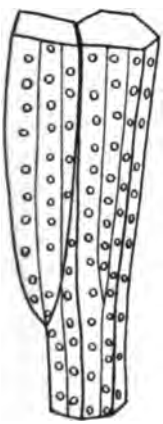

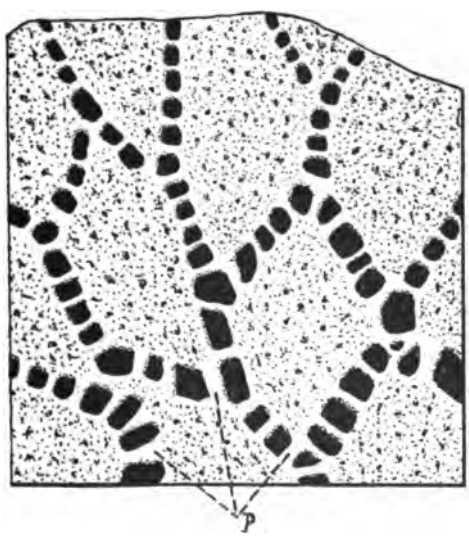


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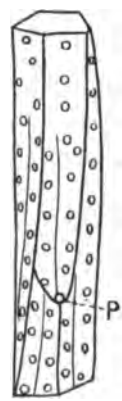

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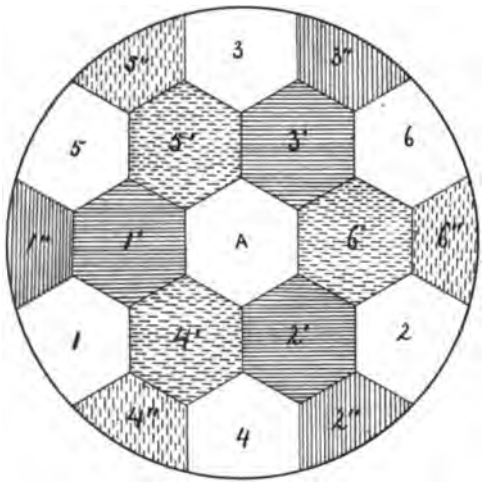
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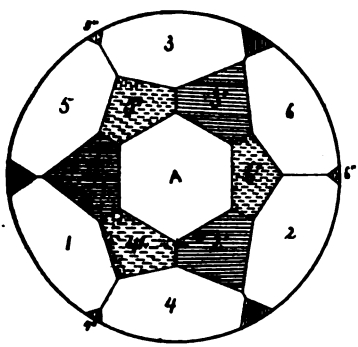
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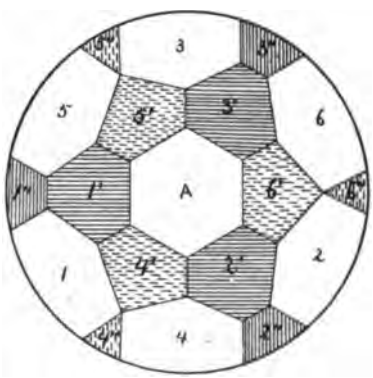
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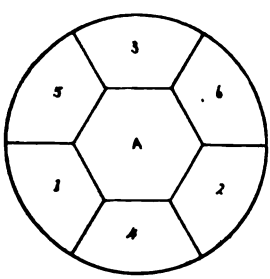
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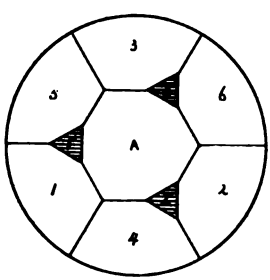
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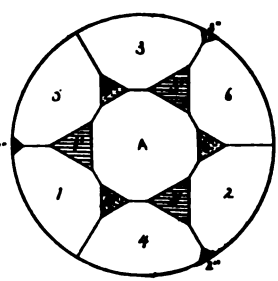
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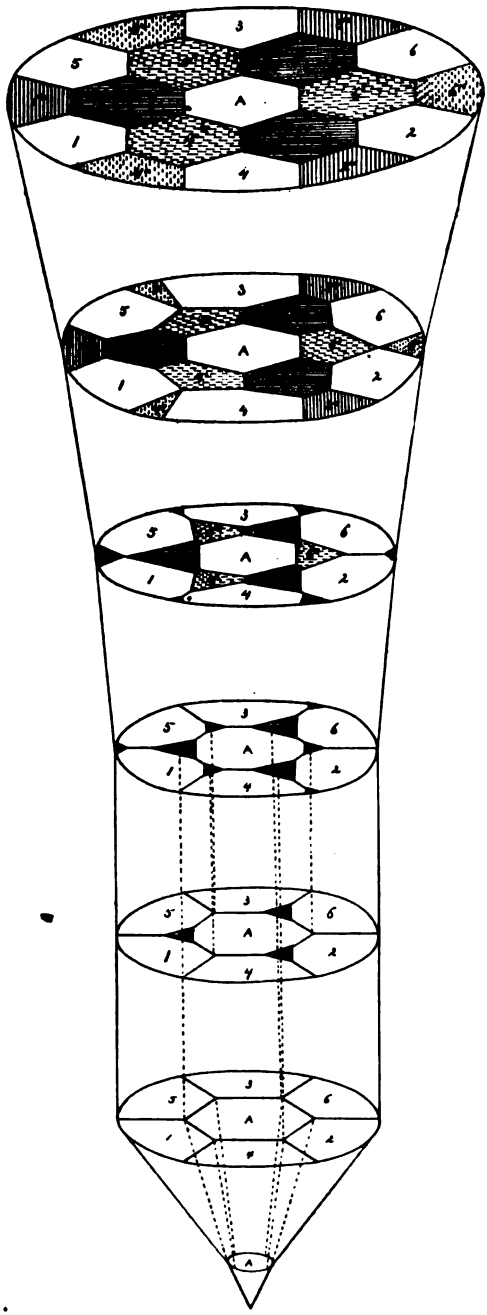

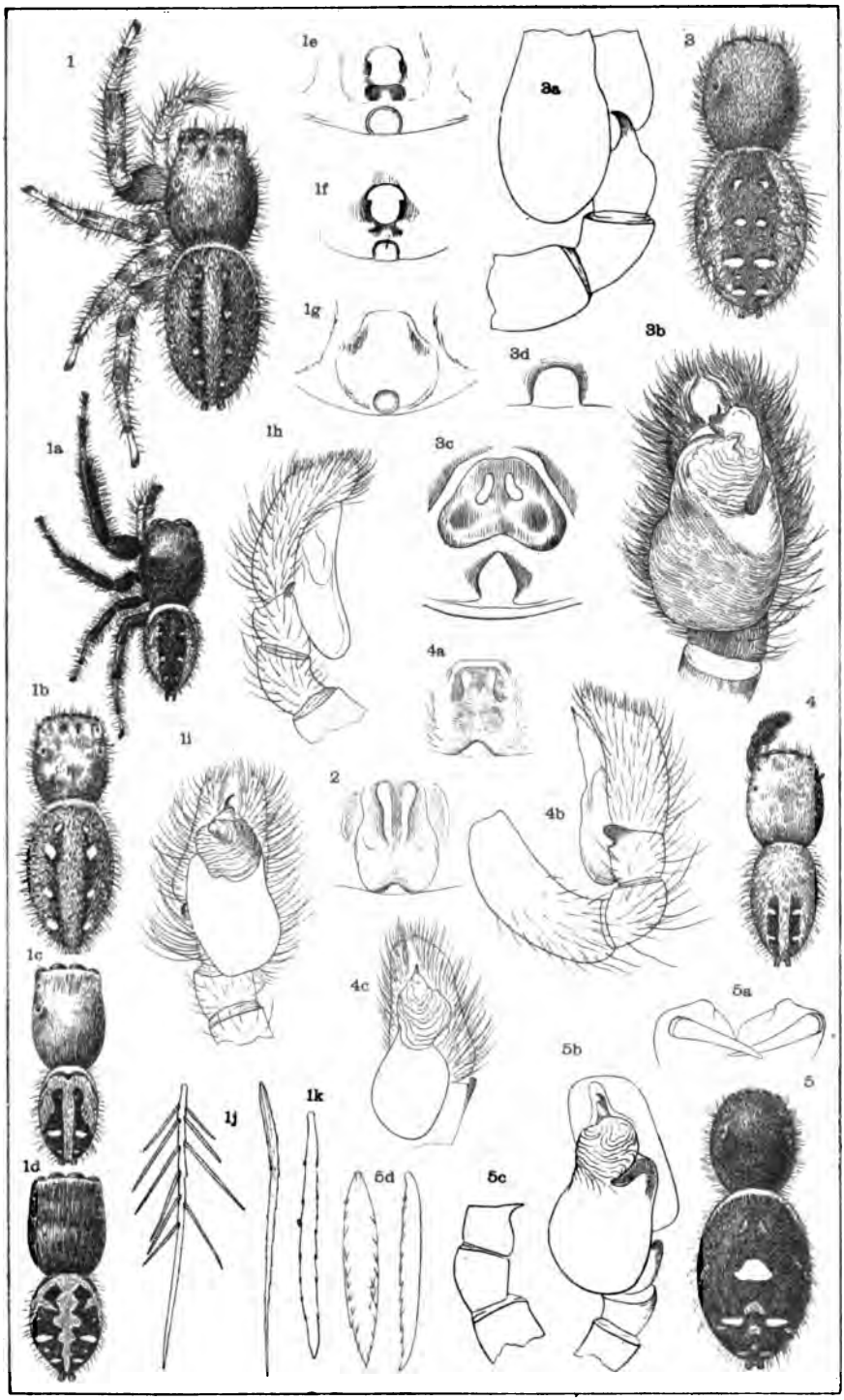


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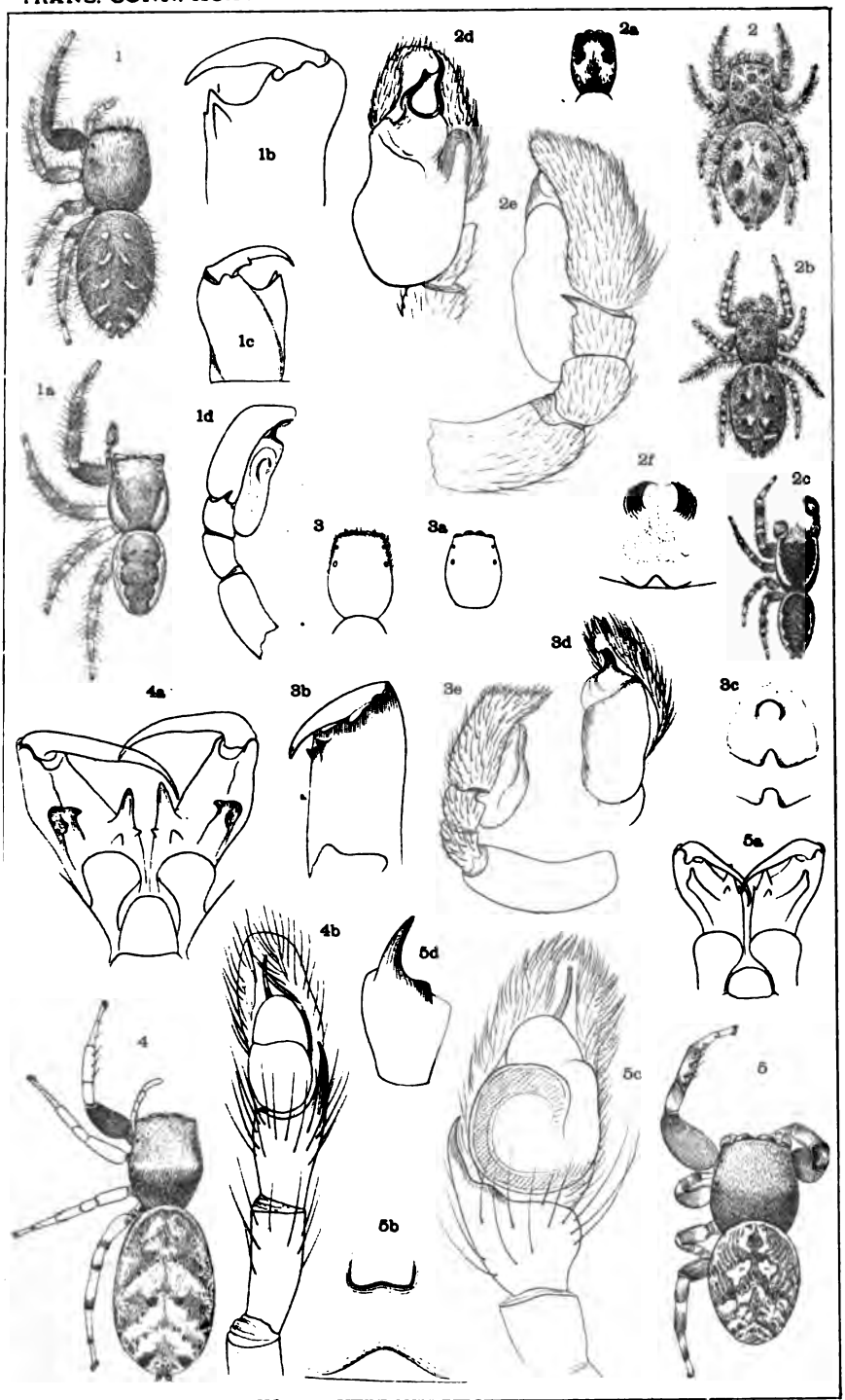


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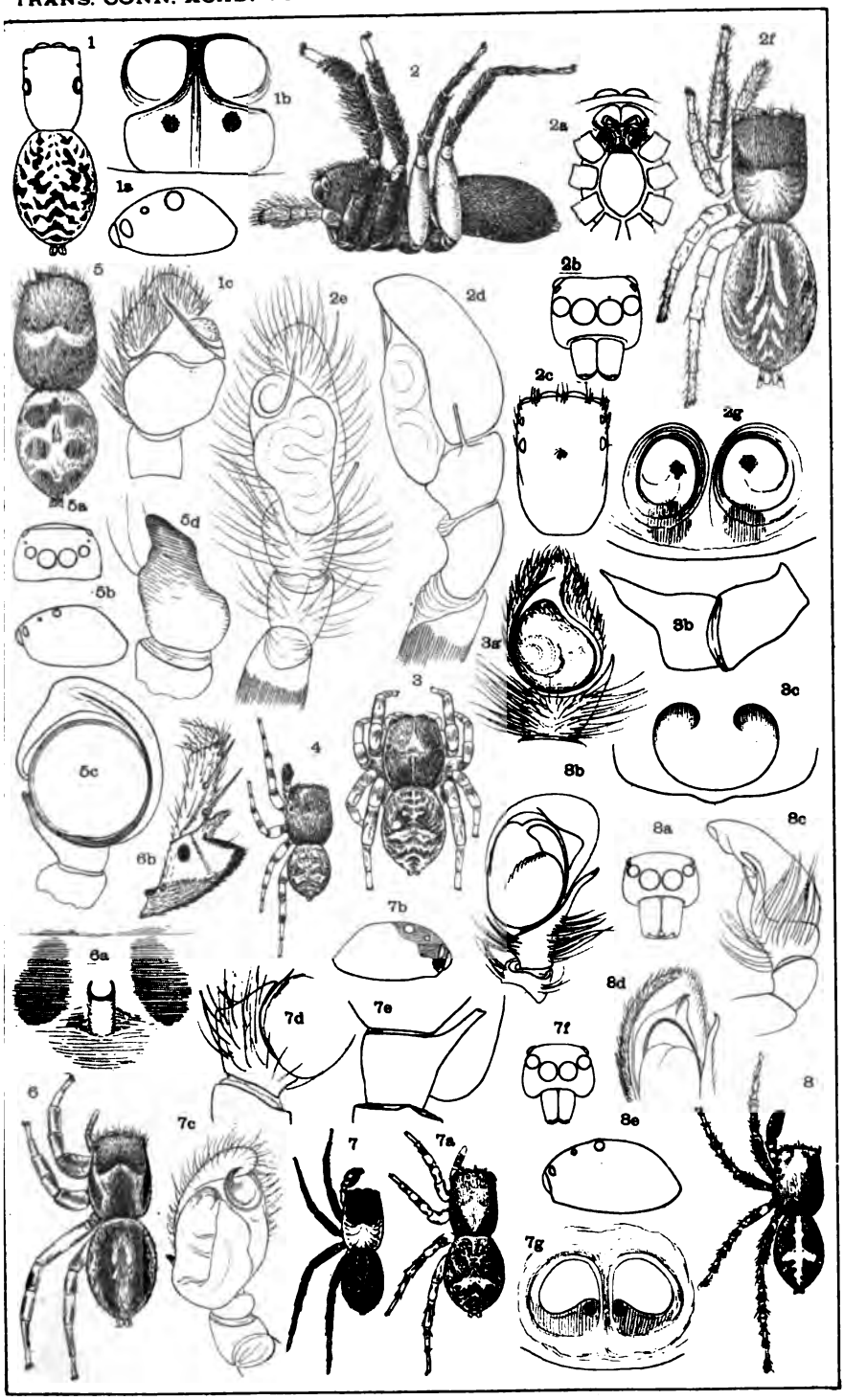


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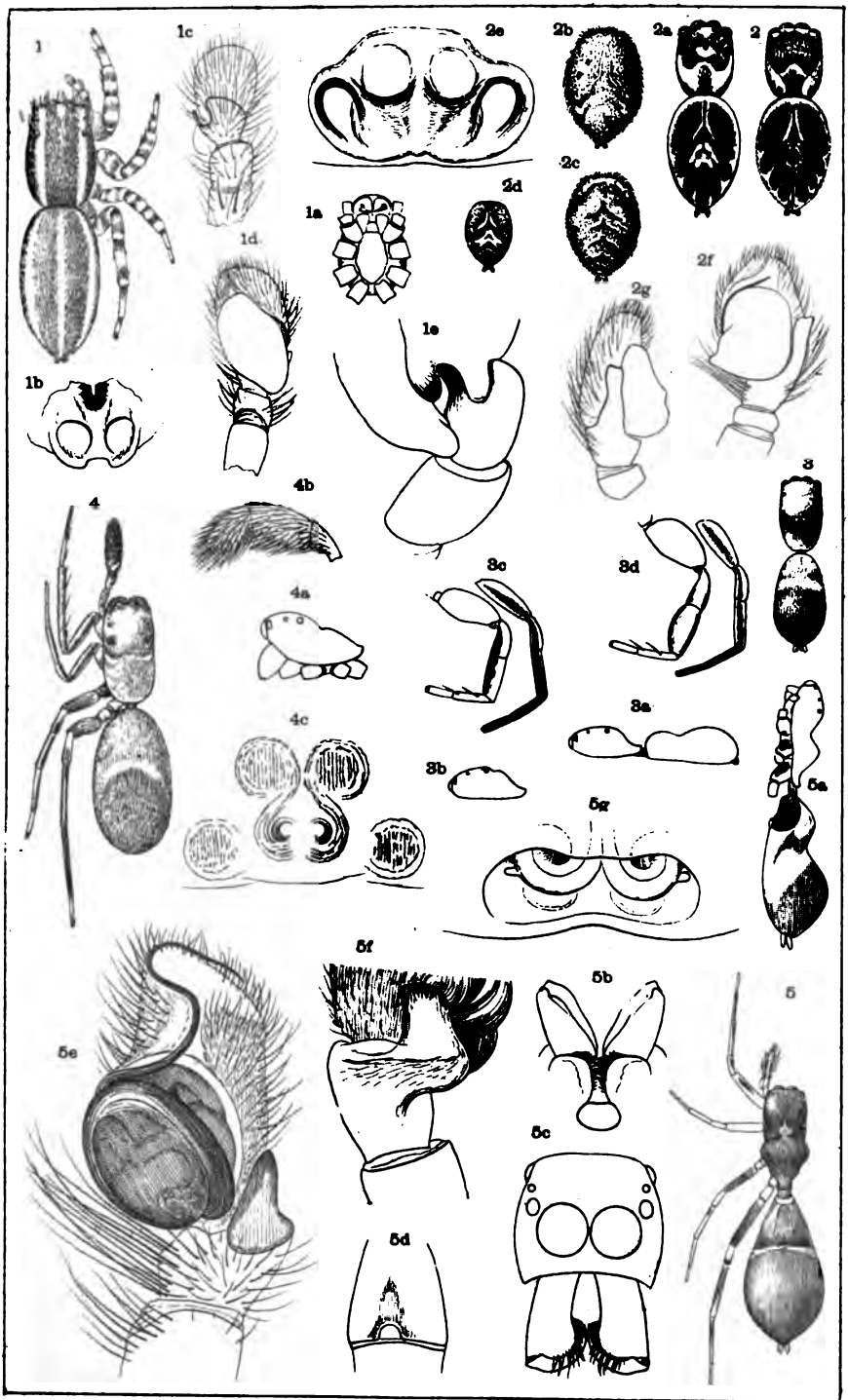


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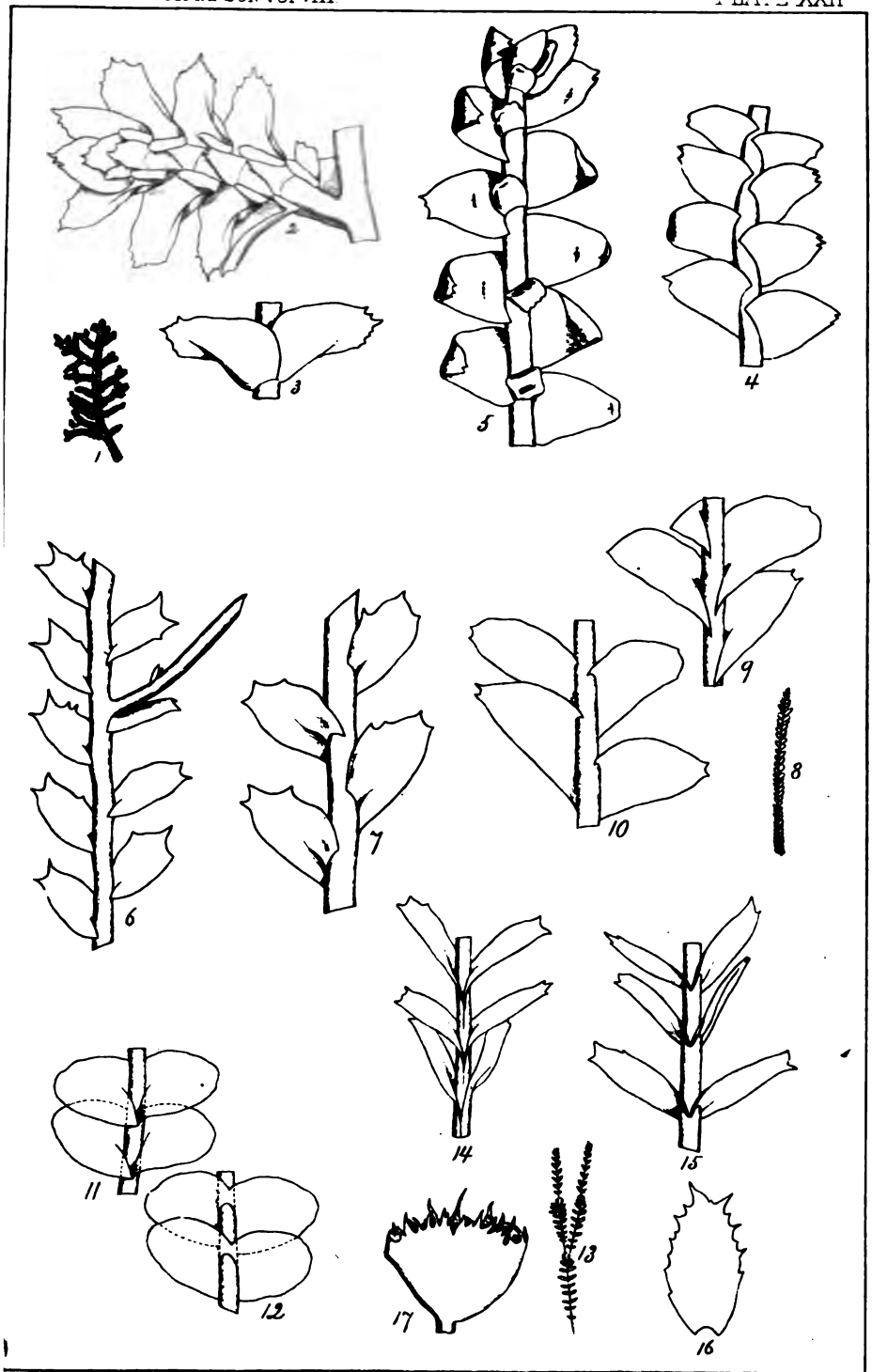


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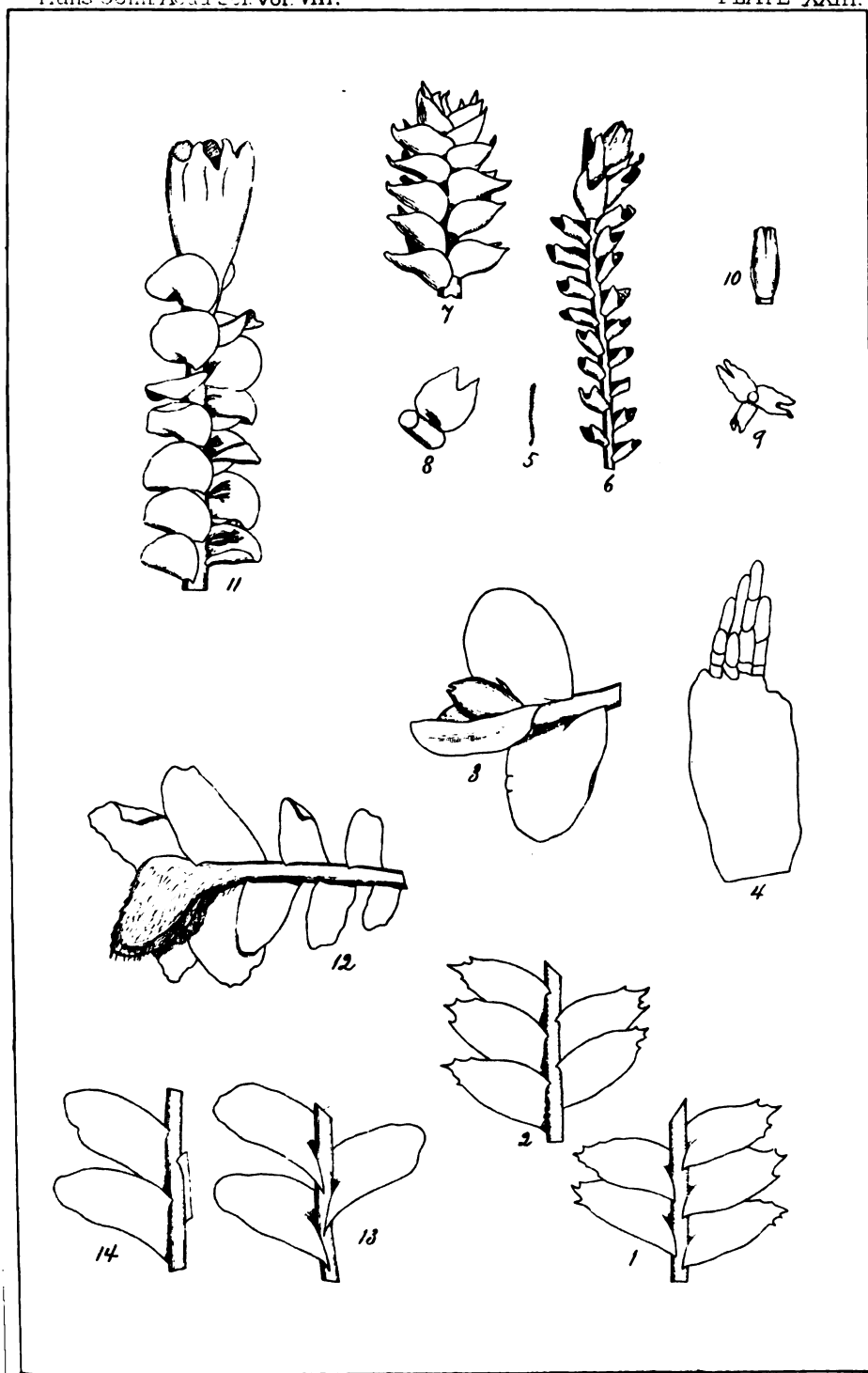


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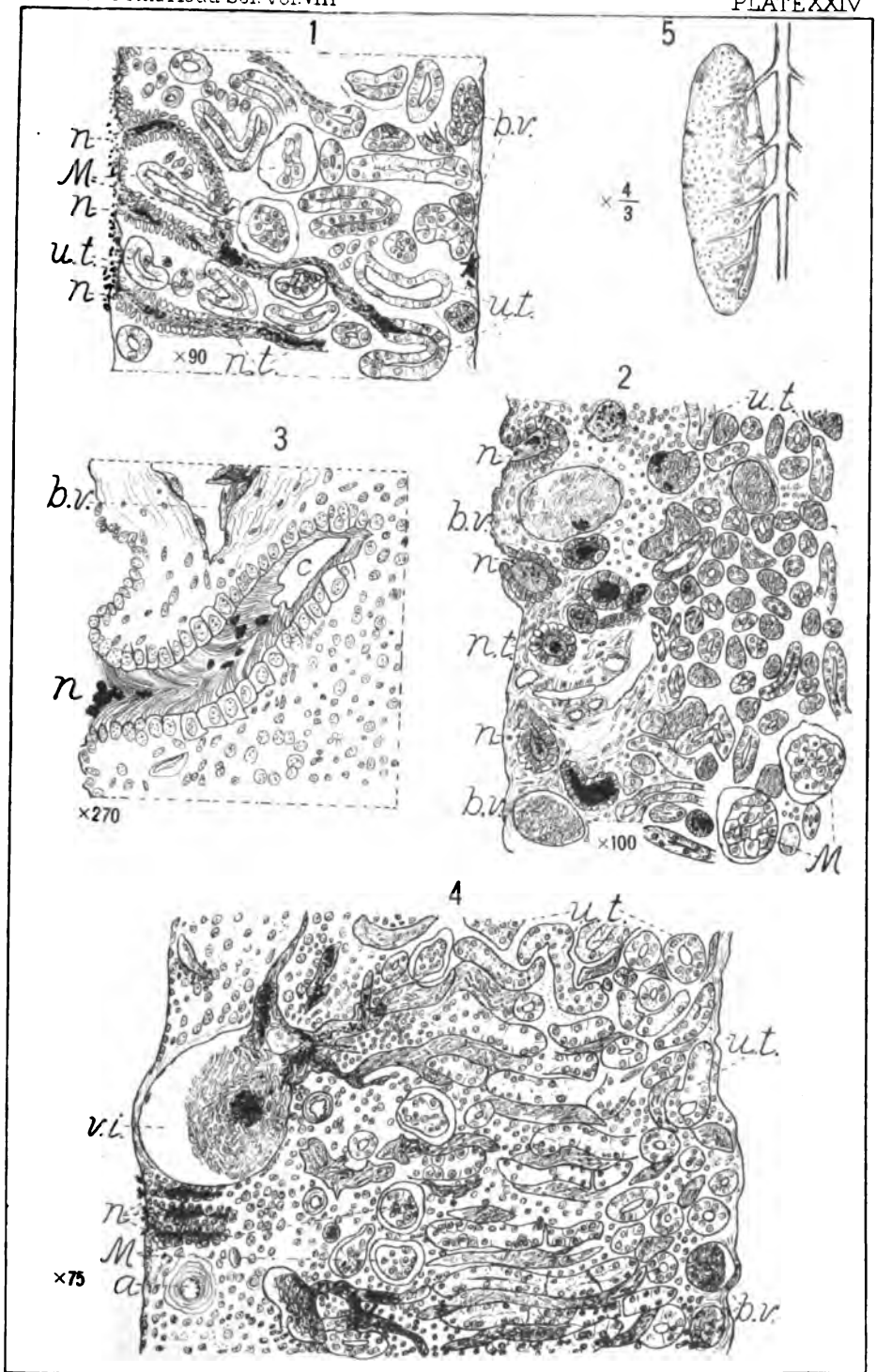


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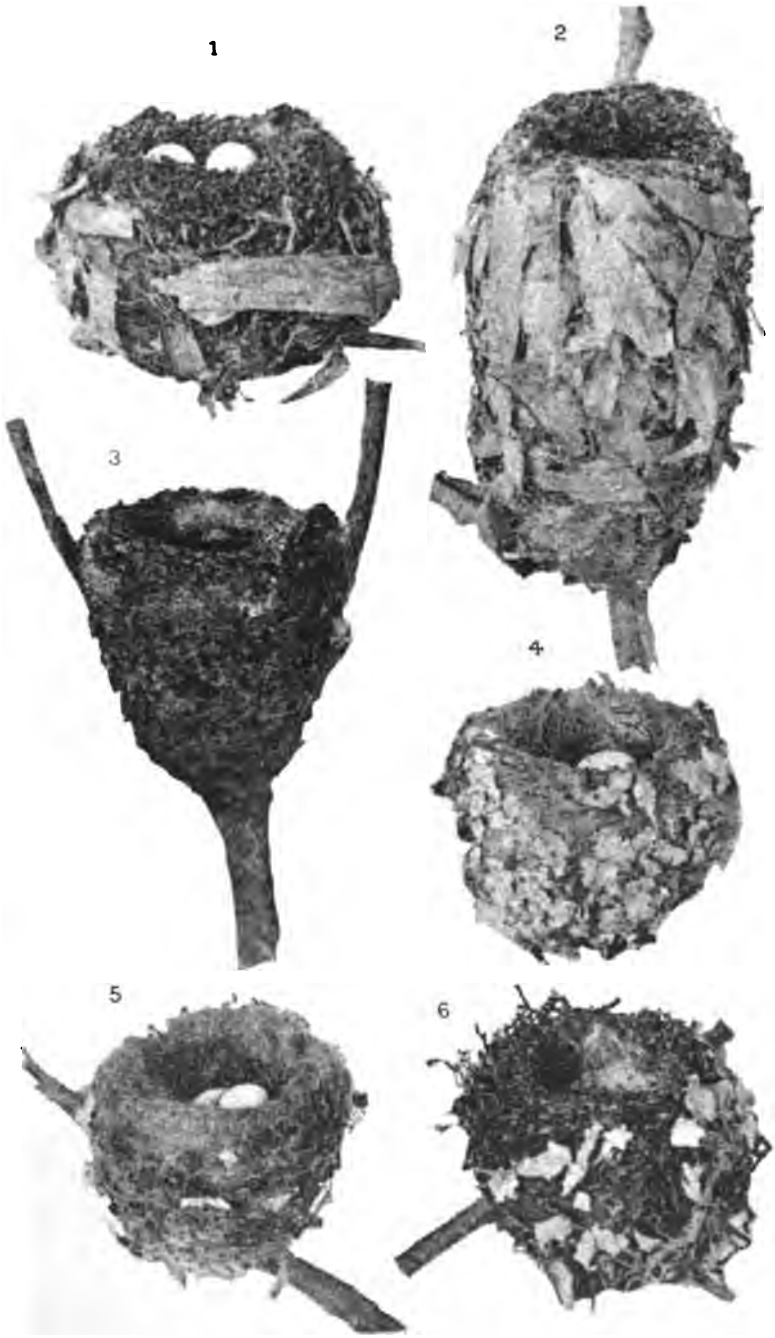


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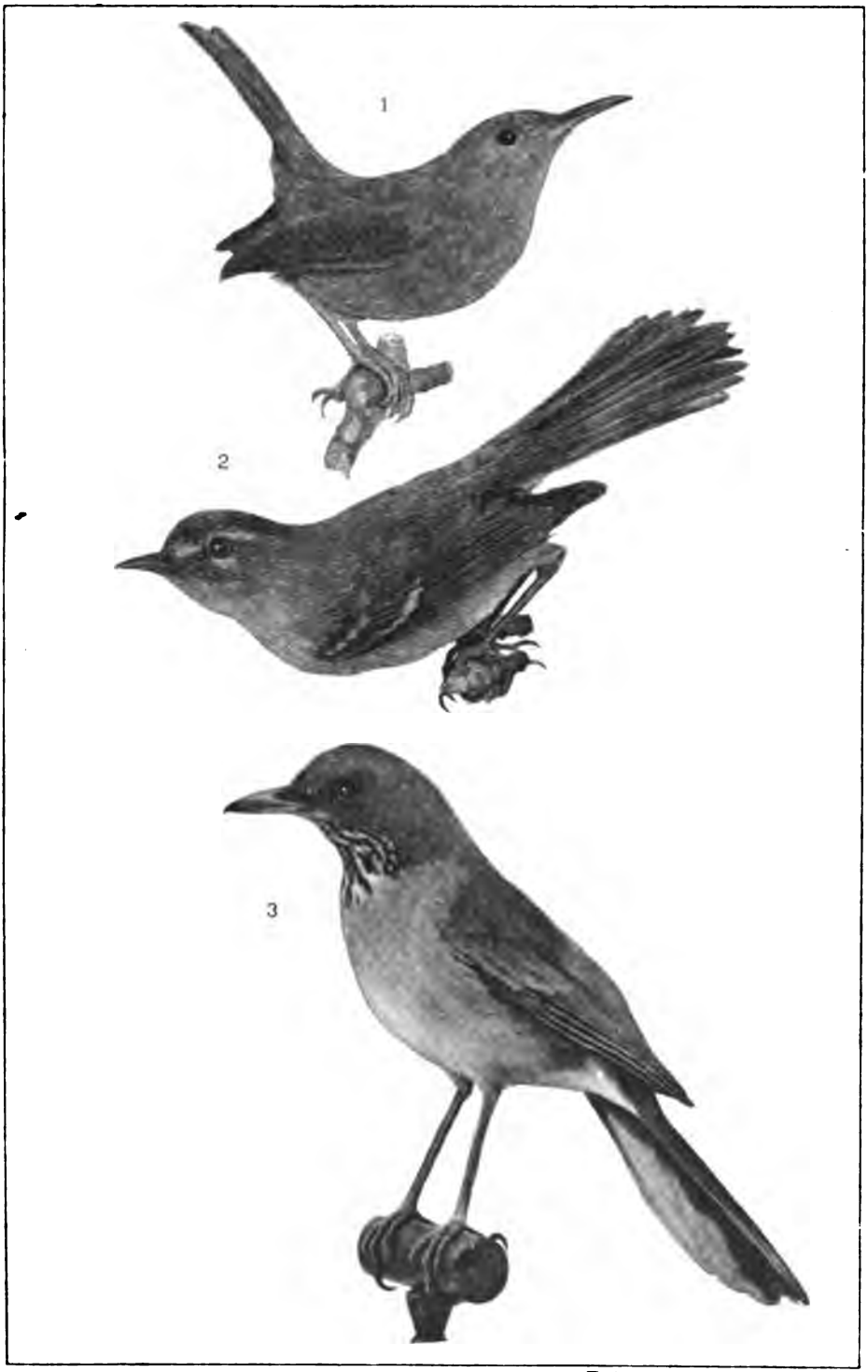


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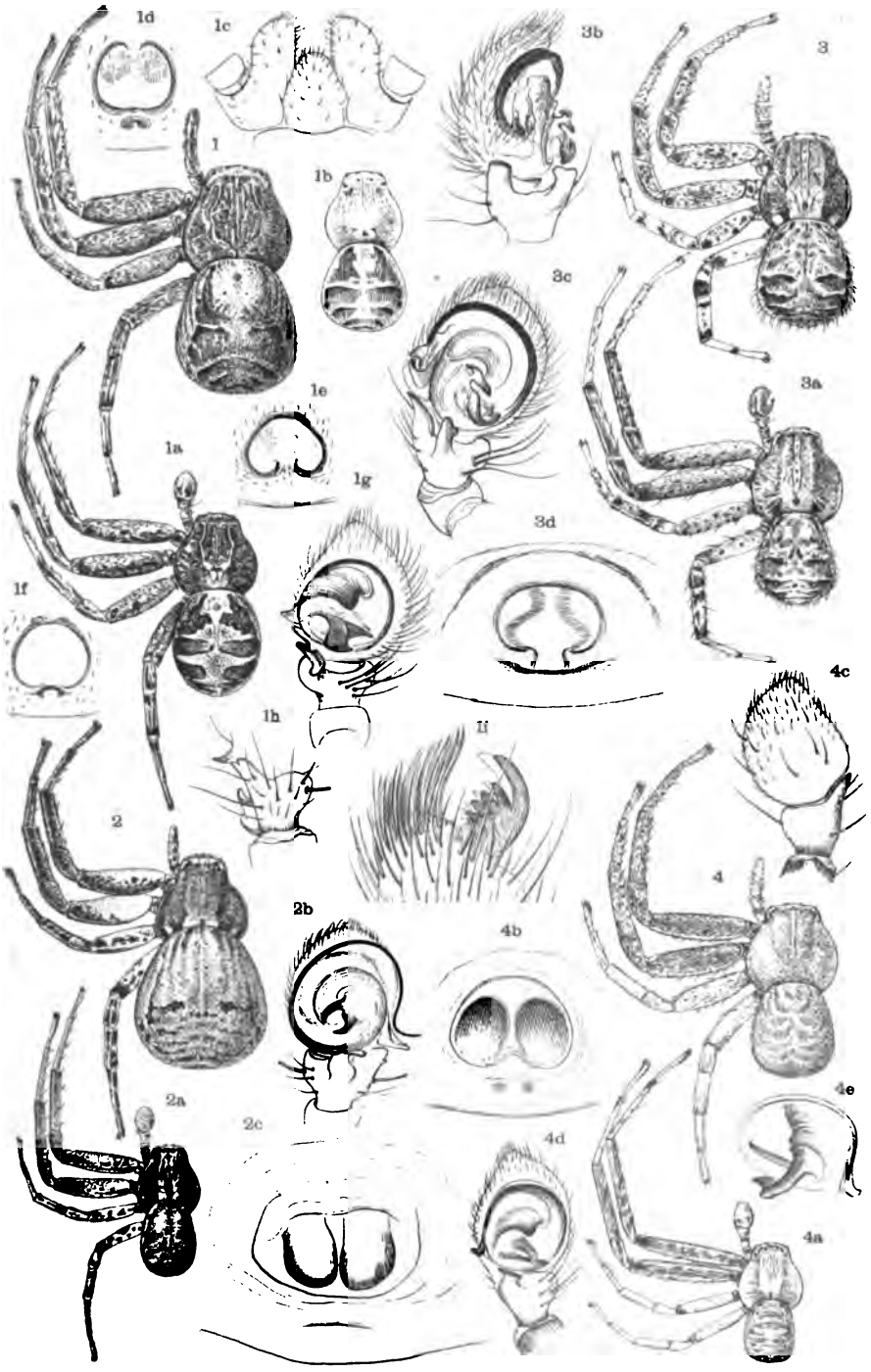


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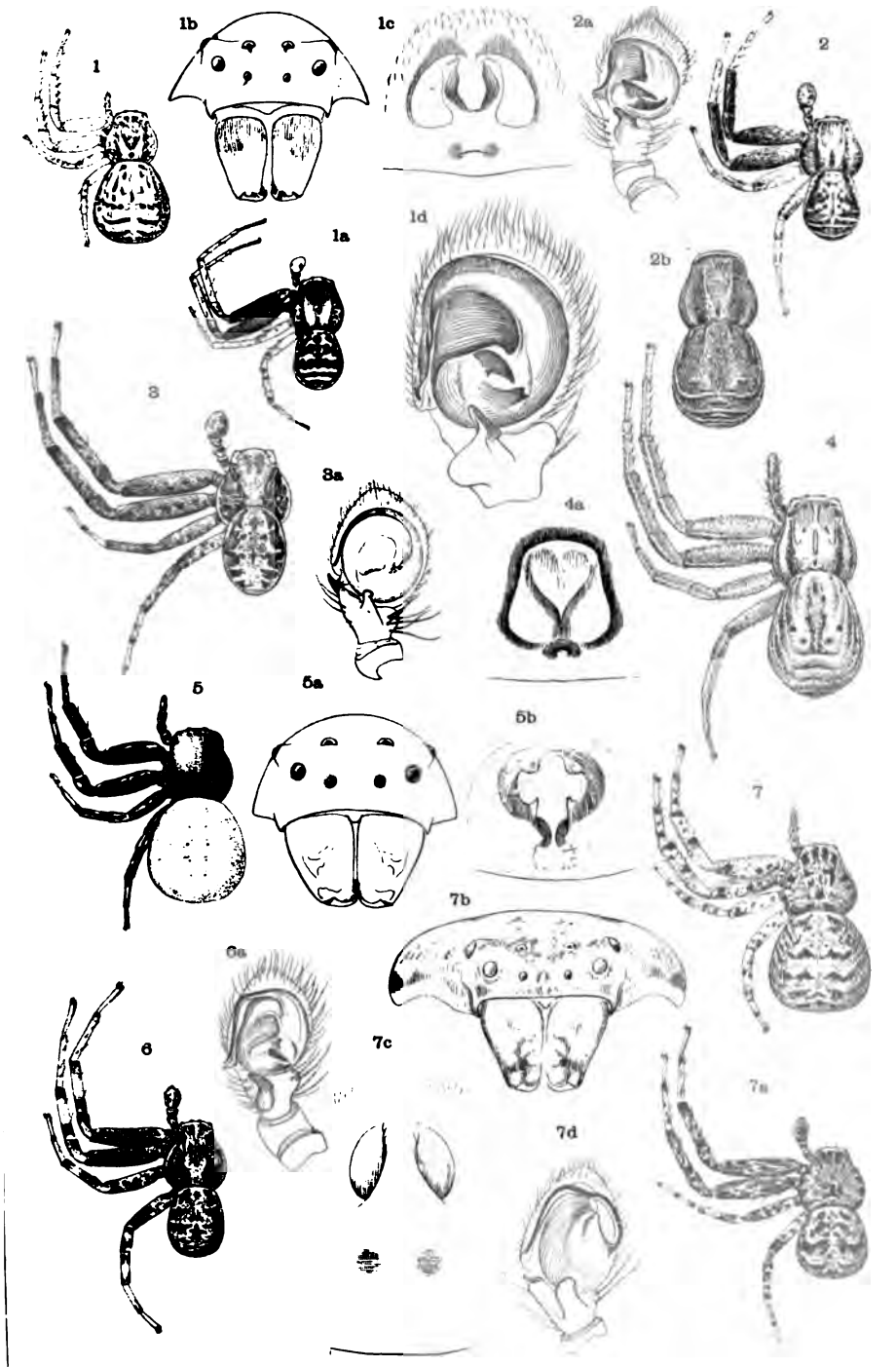


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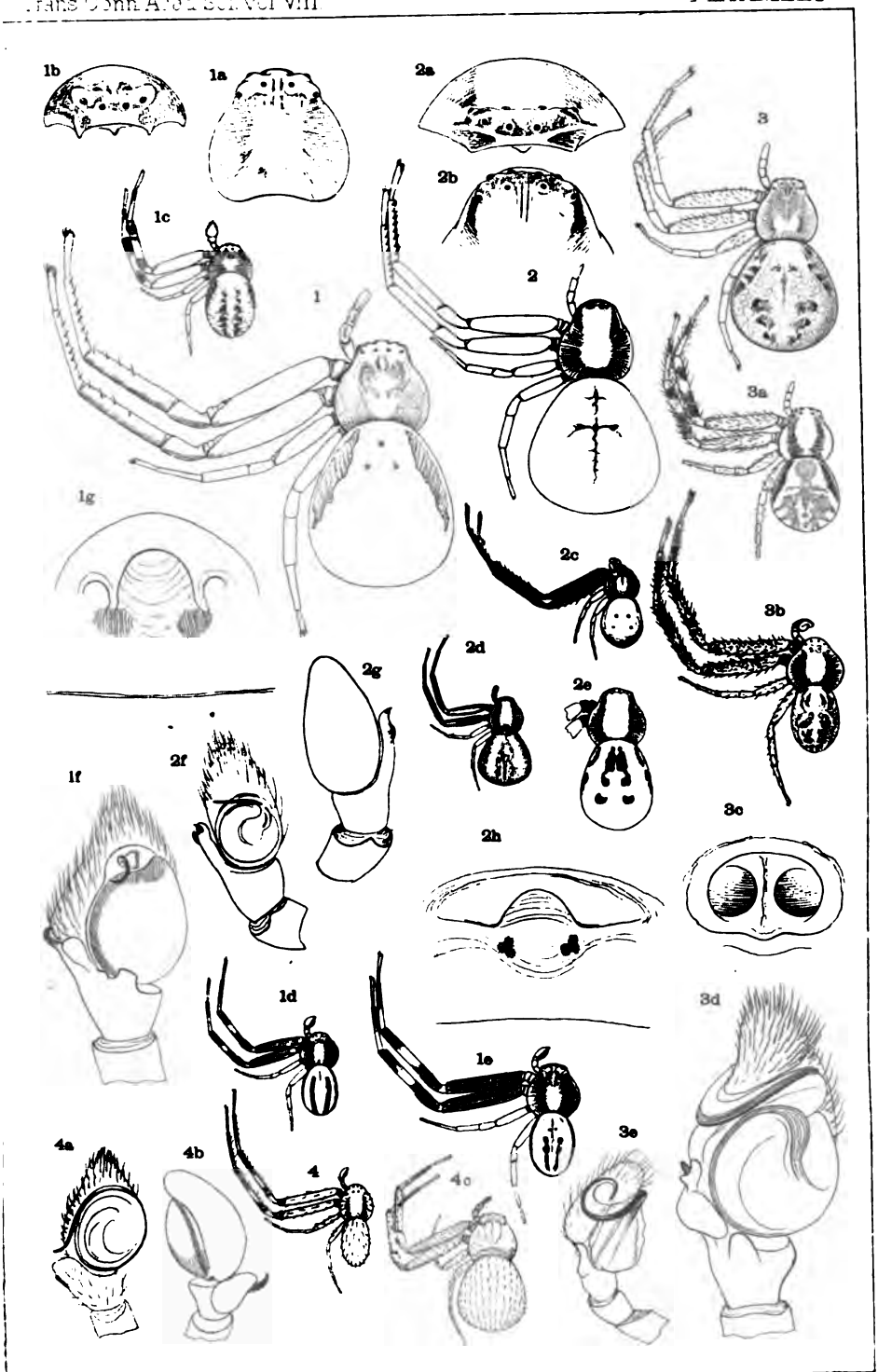


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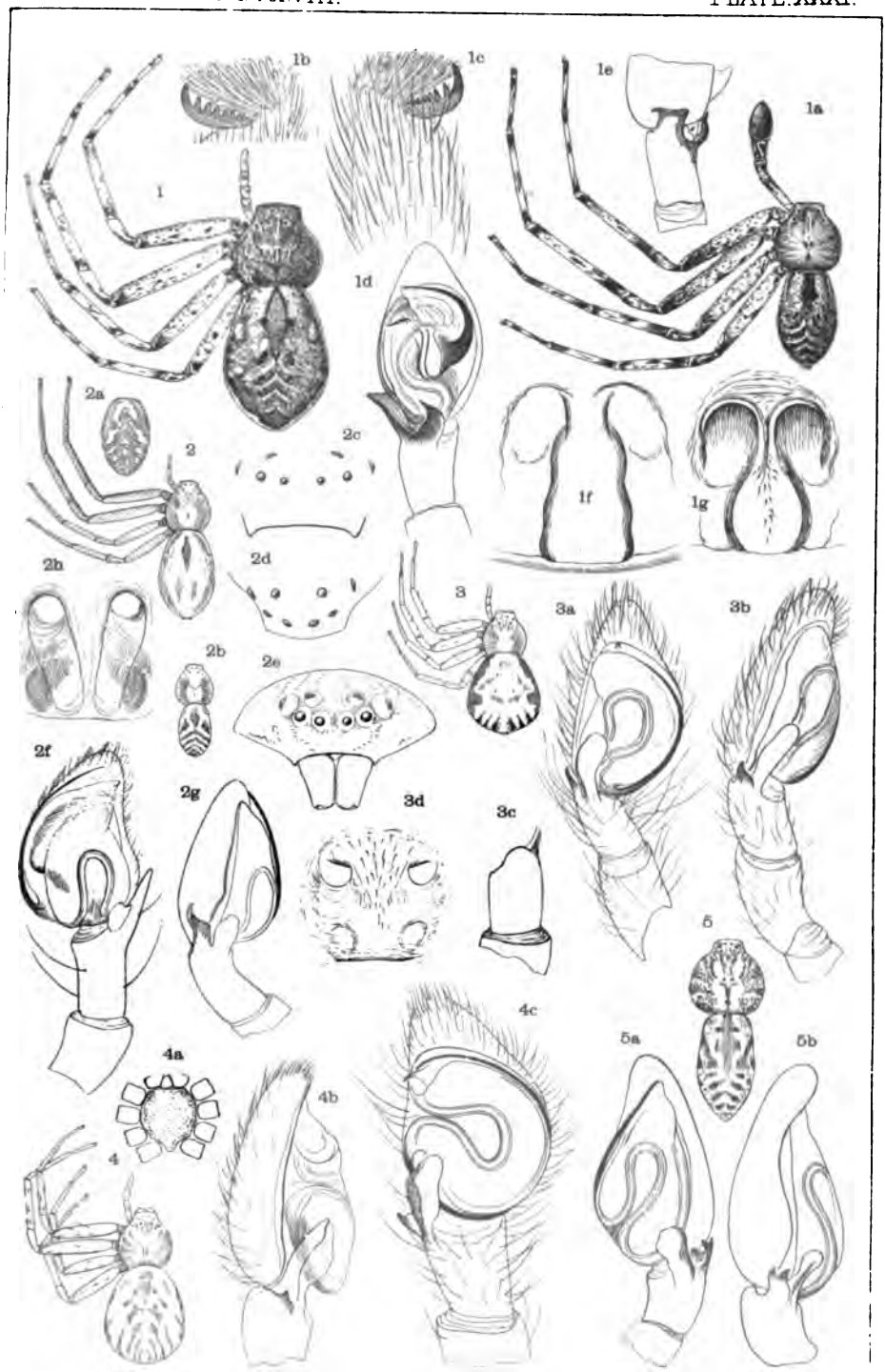


H. Emerton from nature

E. Chisand, lith. New Haven Ct.

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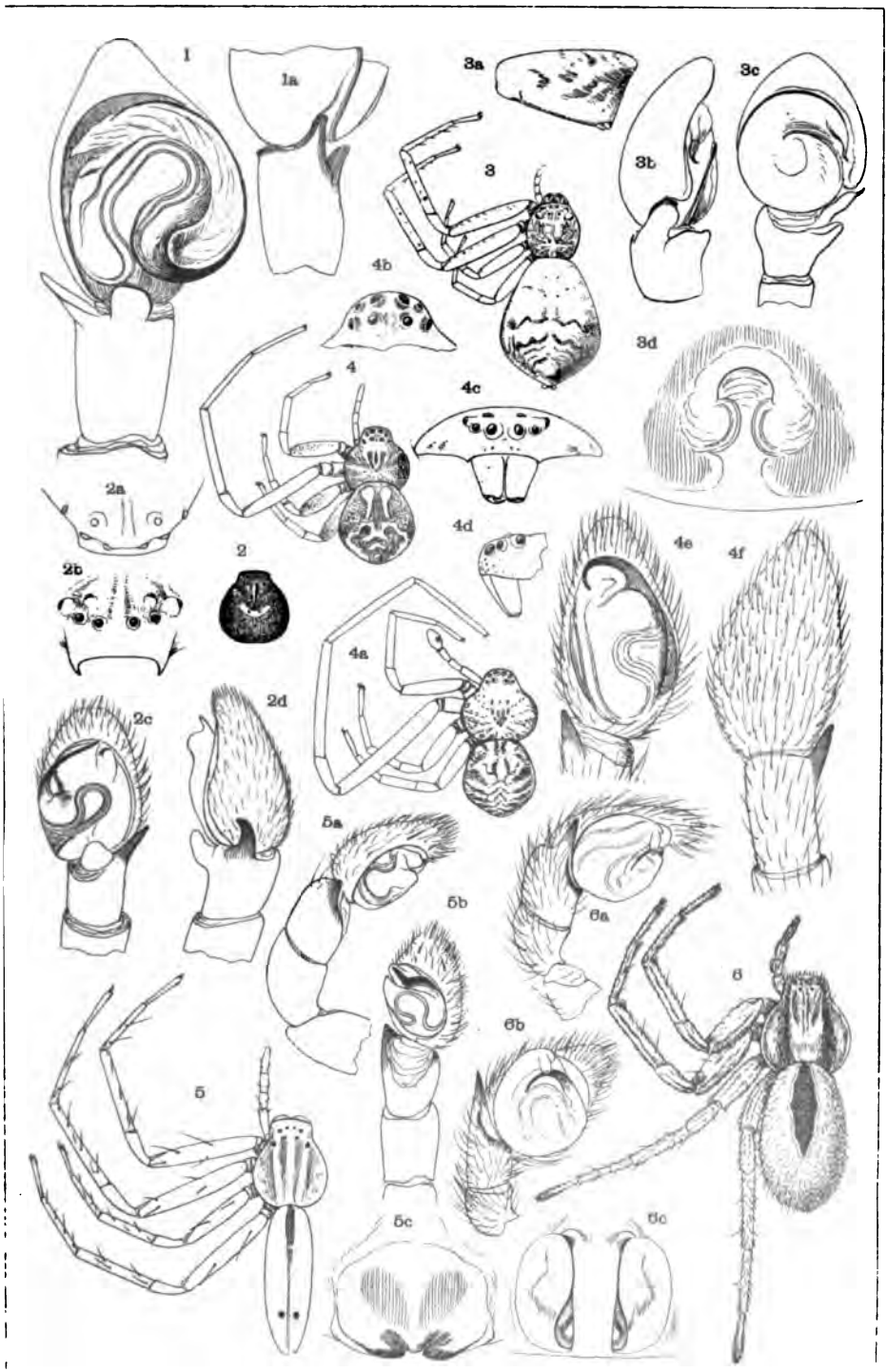


J. H. Emerton, from nature.

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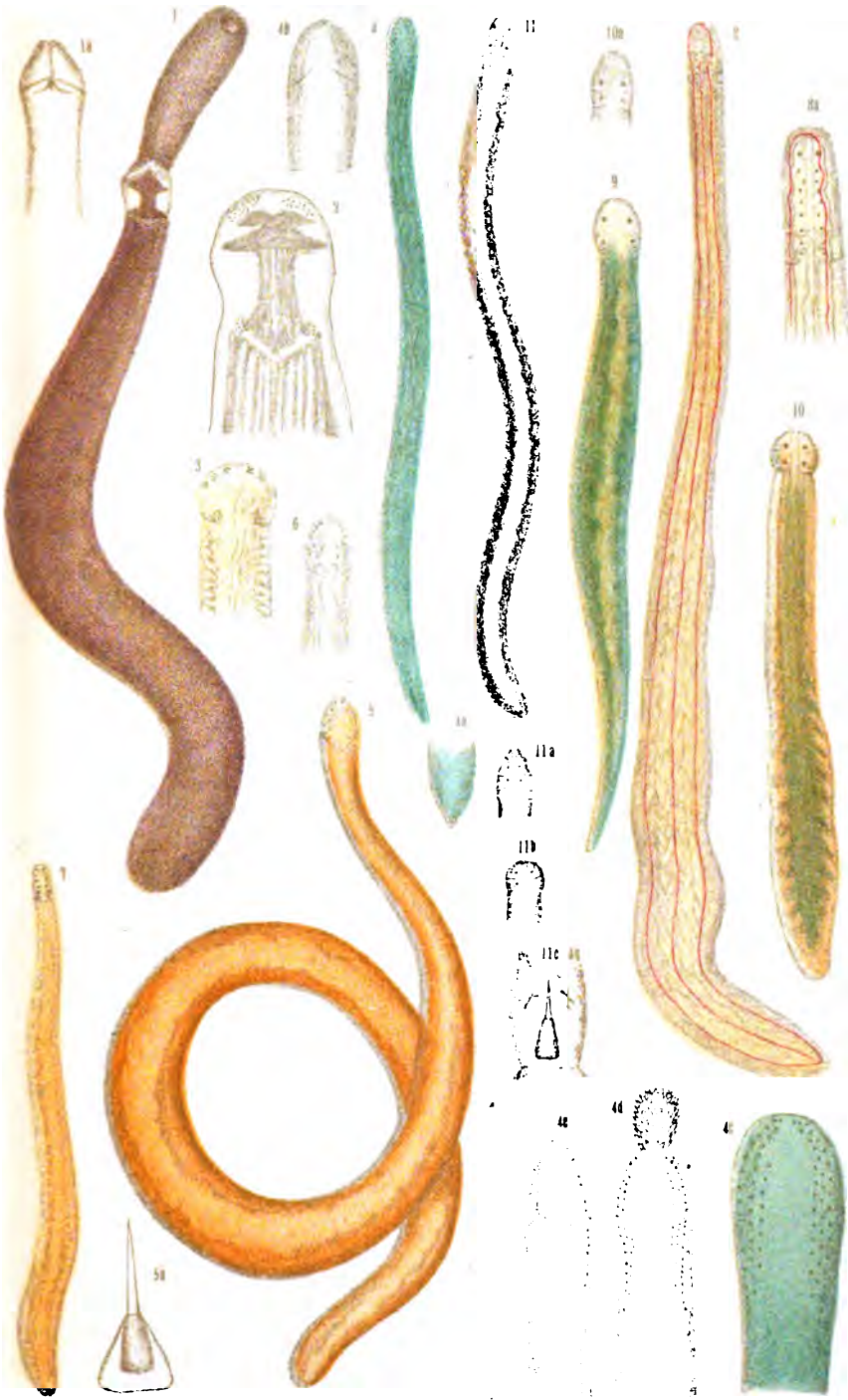

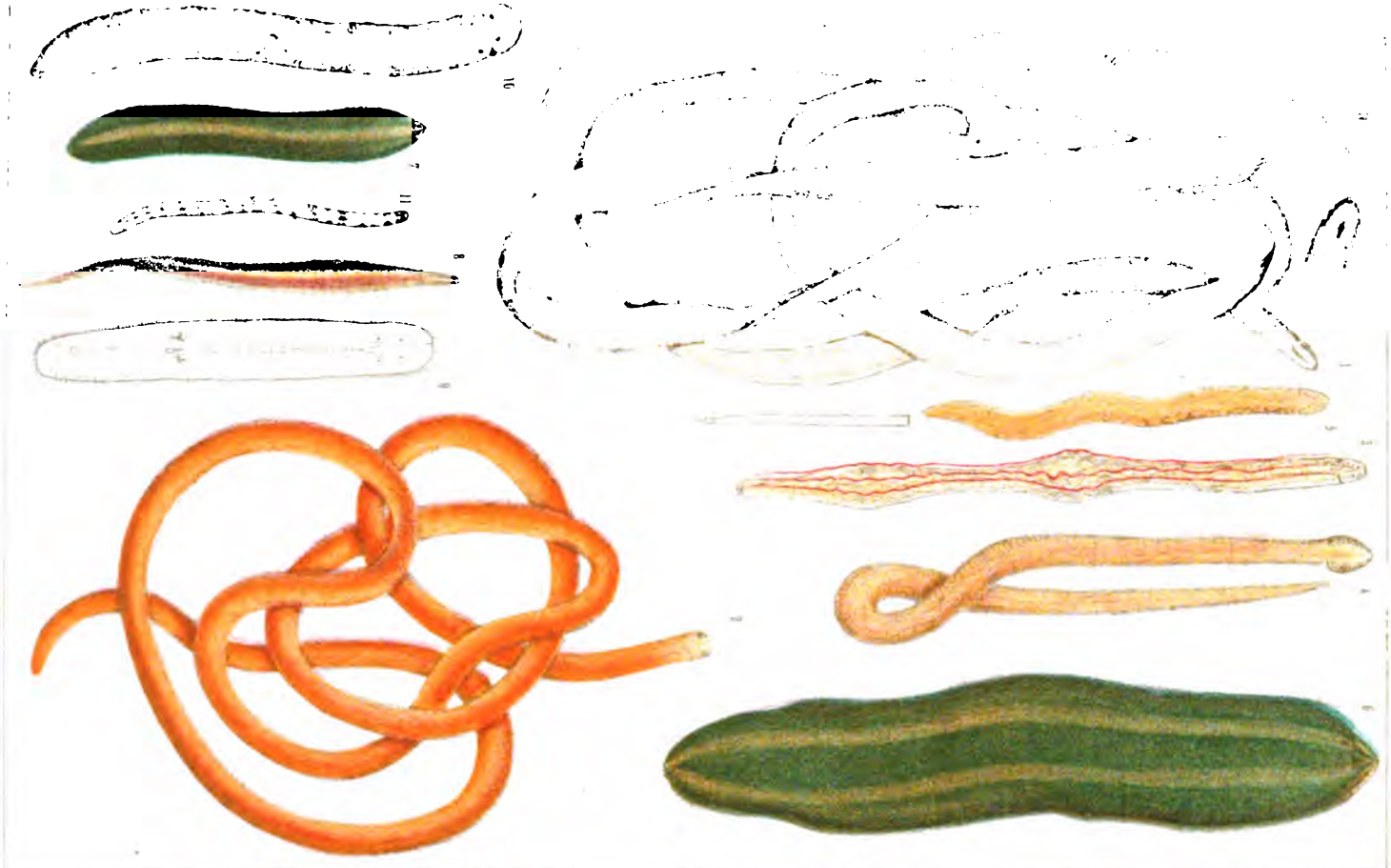

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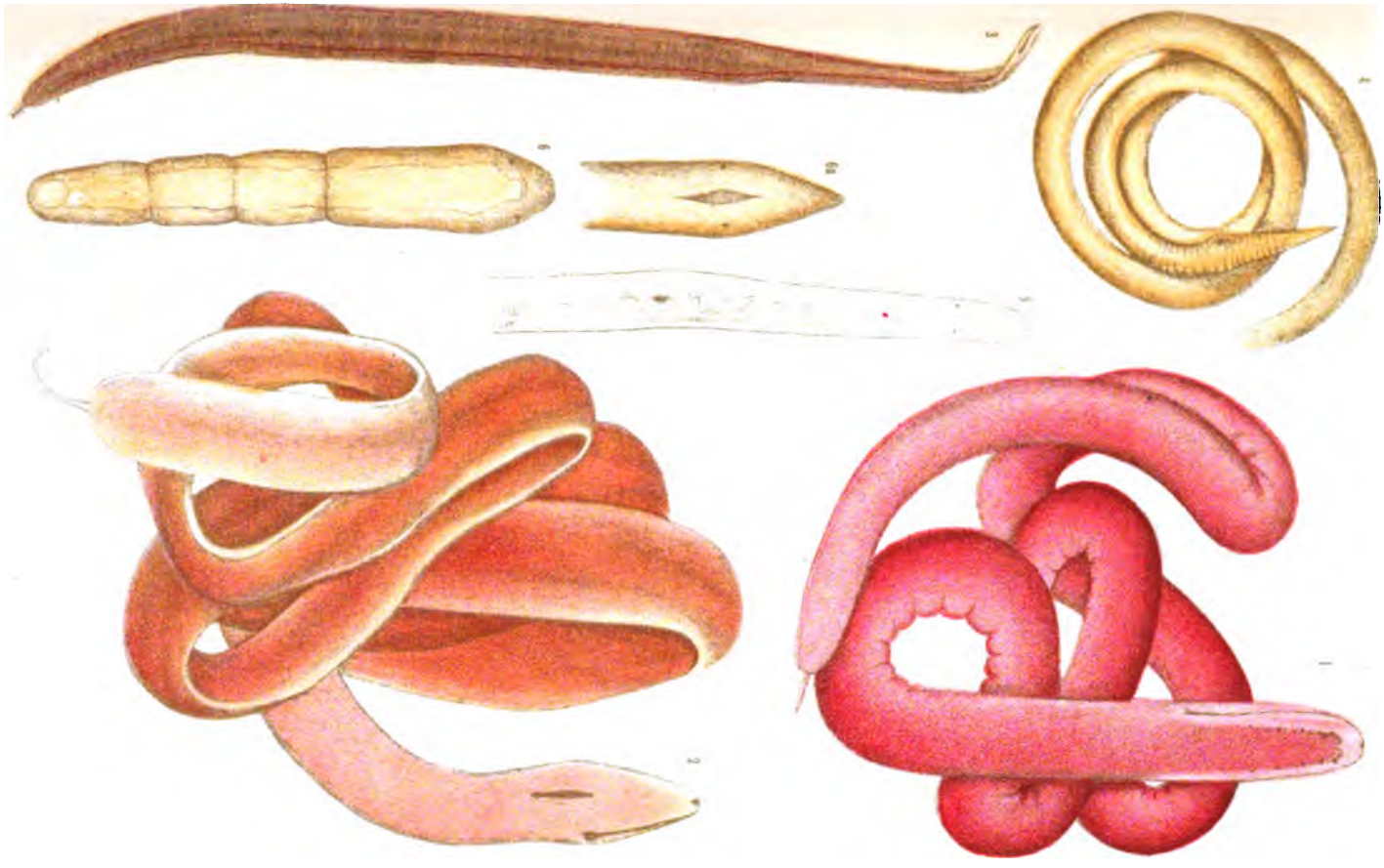
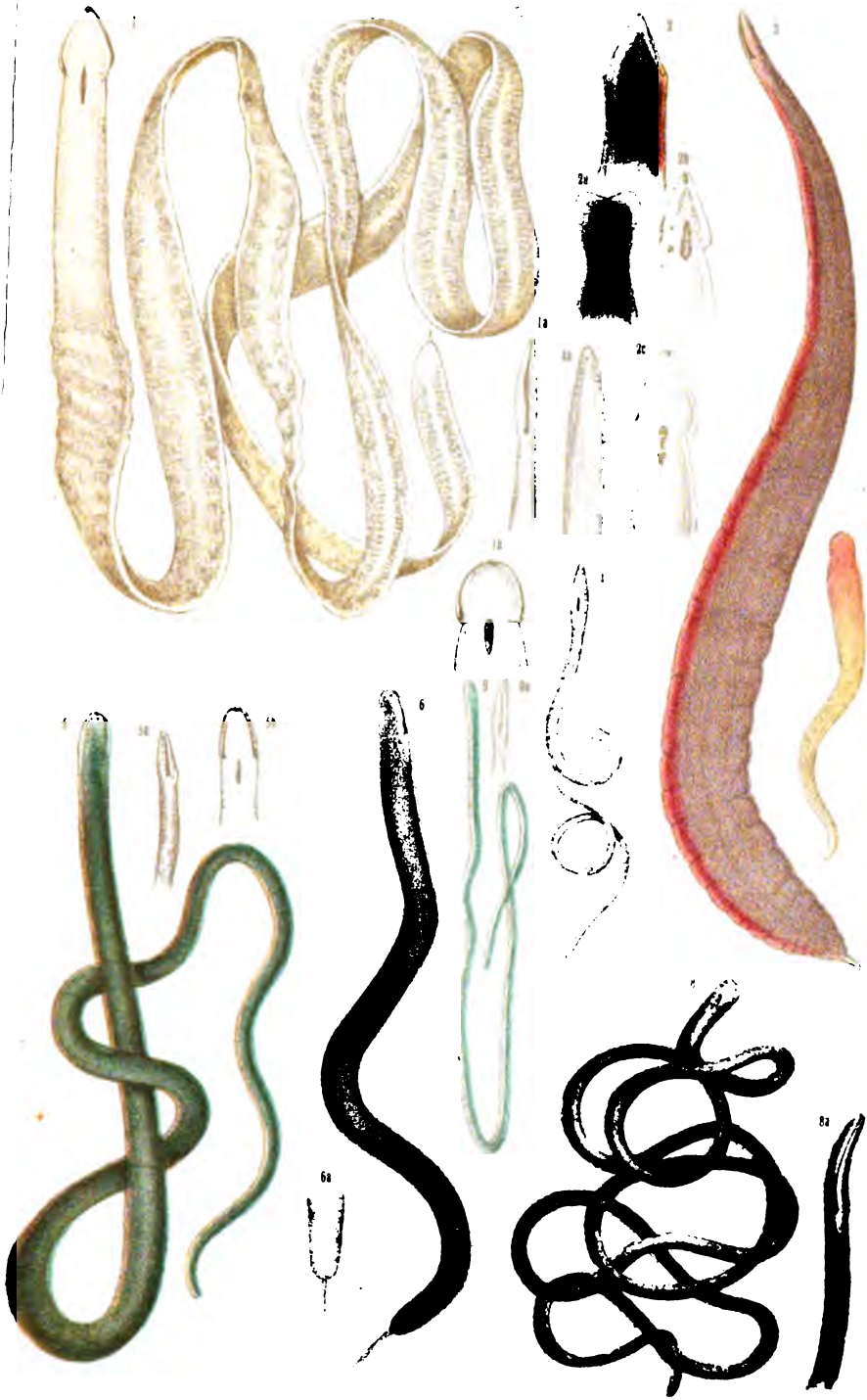
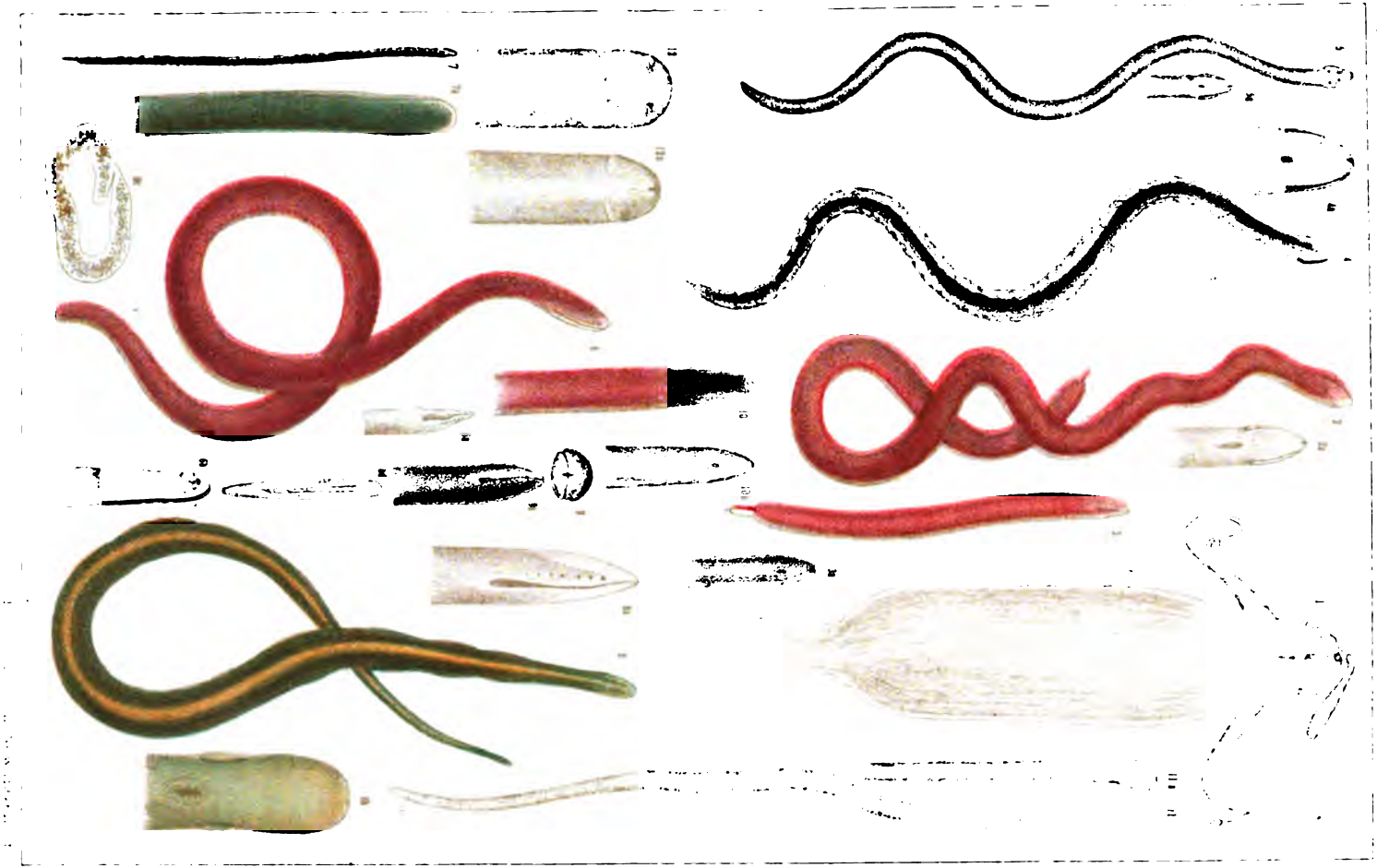
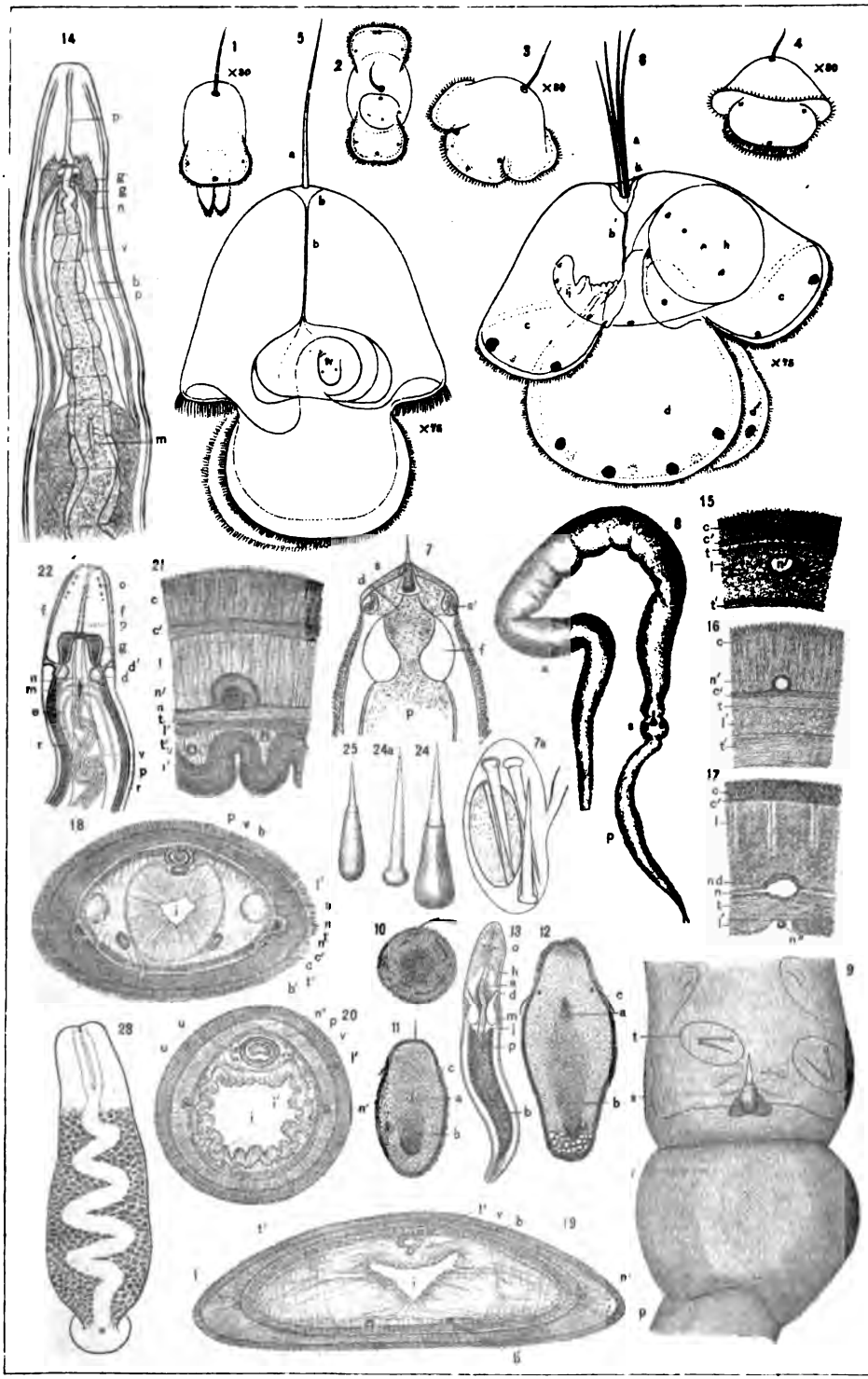


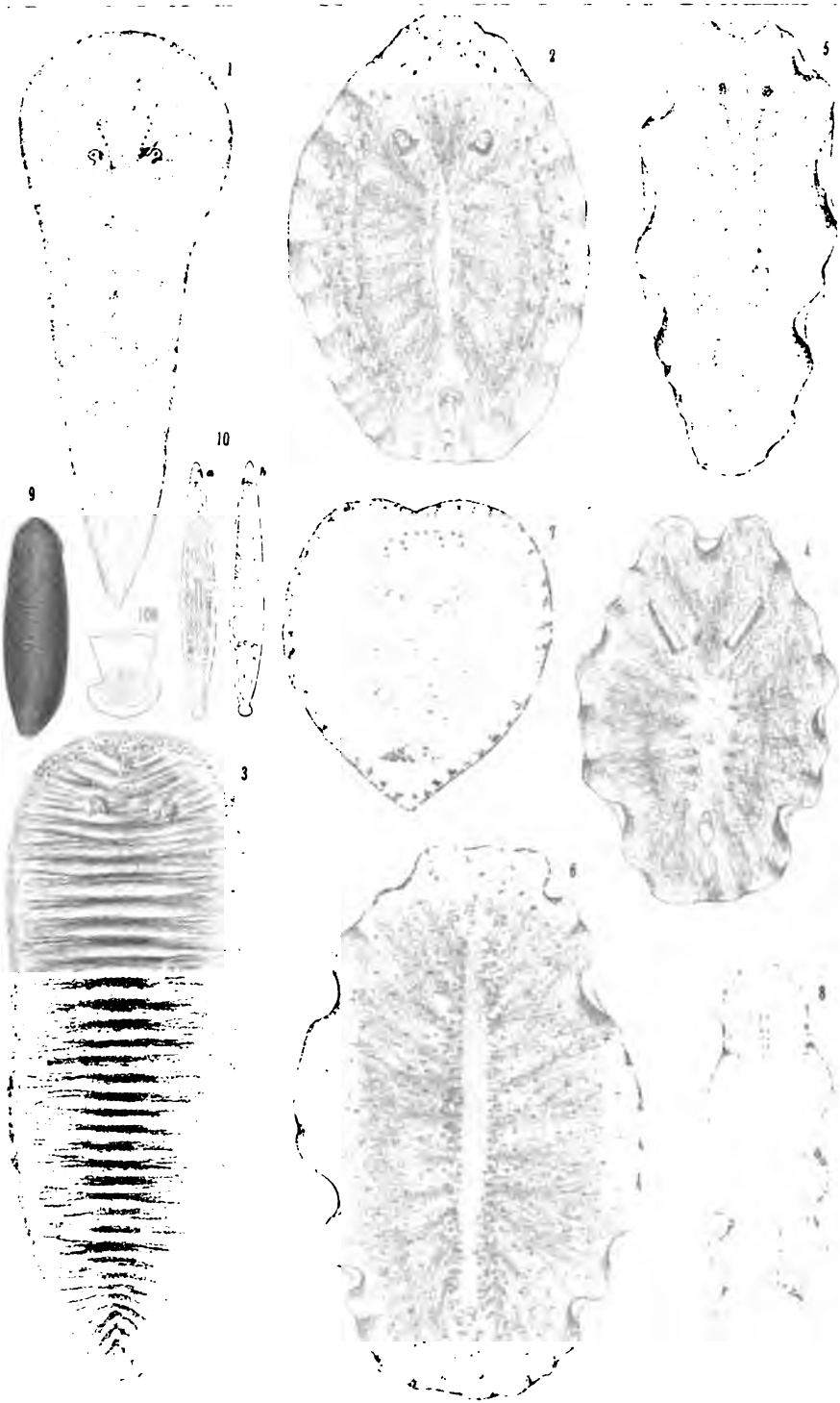






E. Chisand, lith., New Haven Ct.

NEMERTEANS.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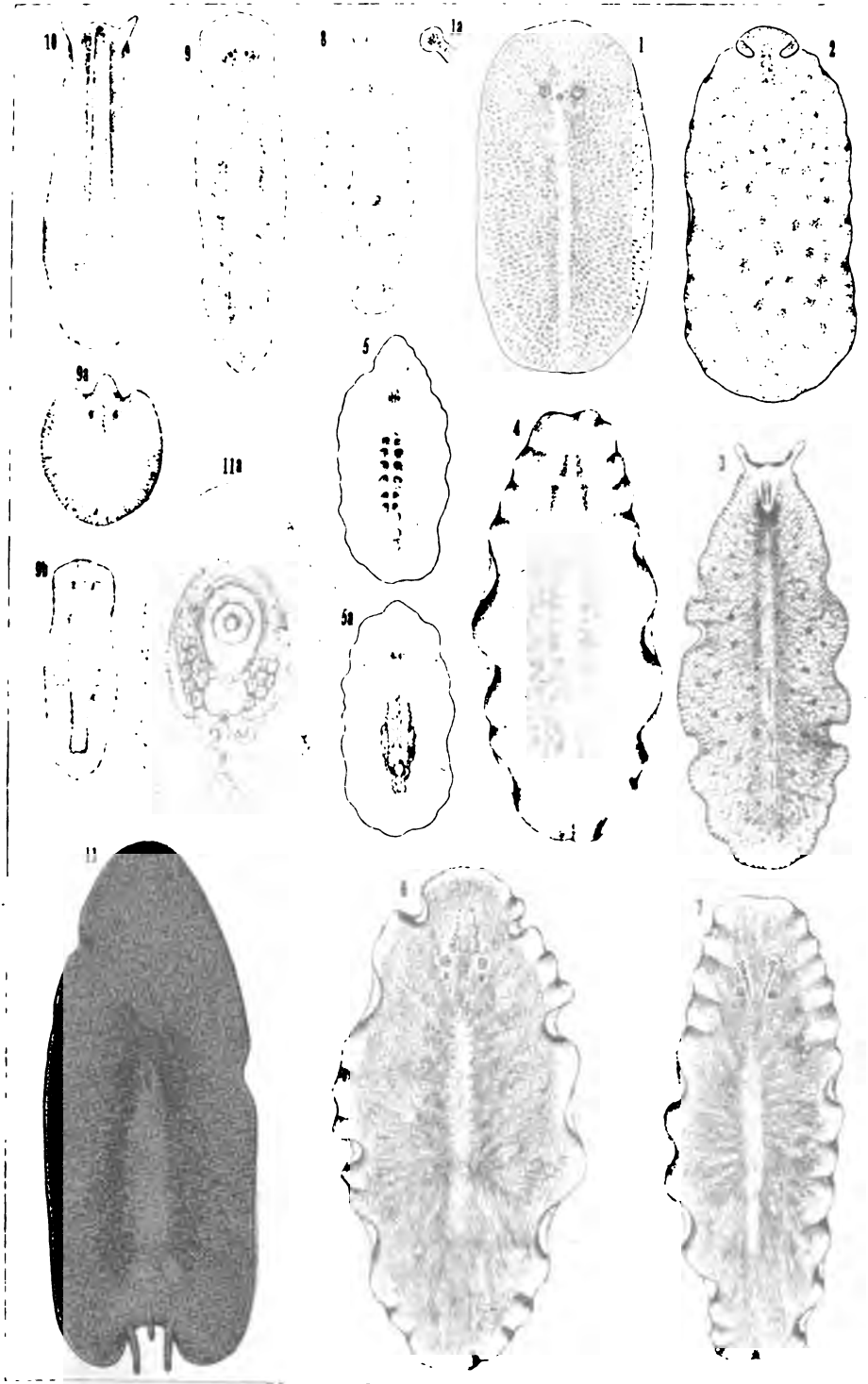
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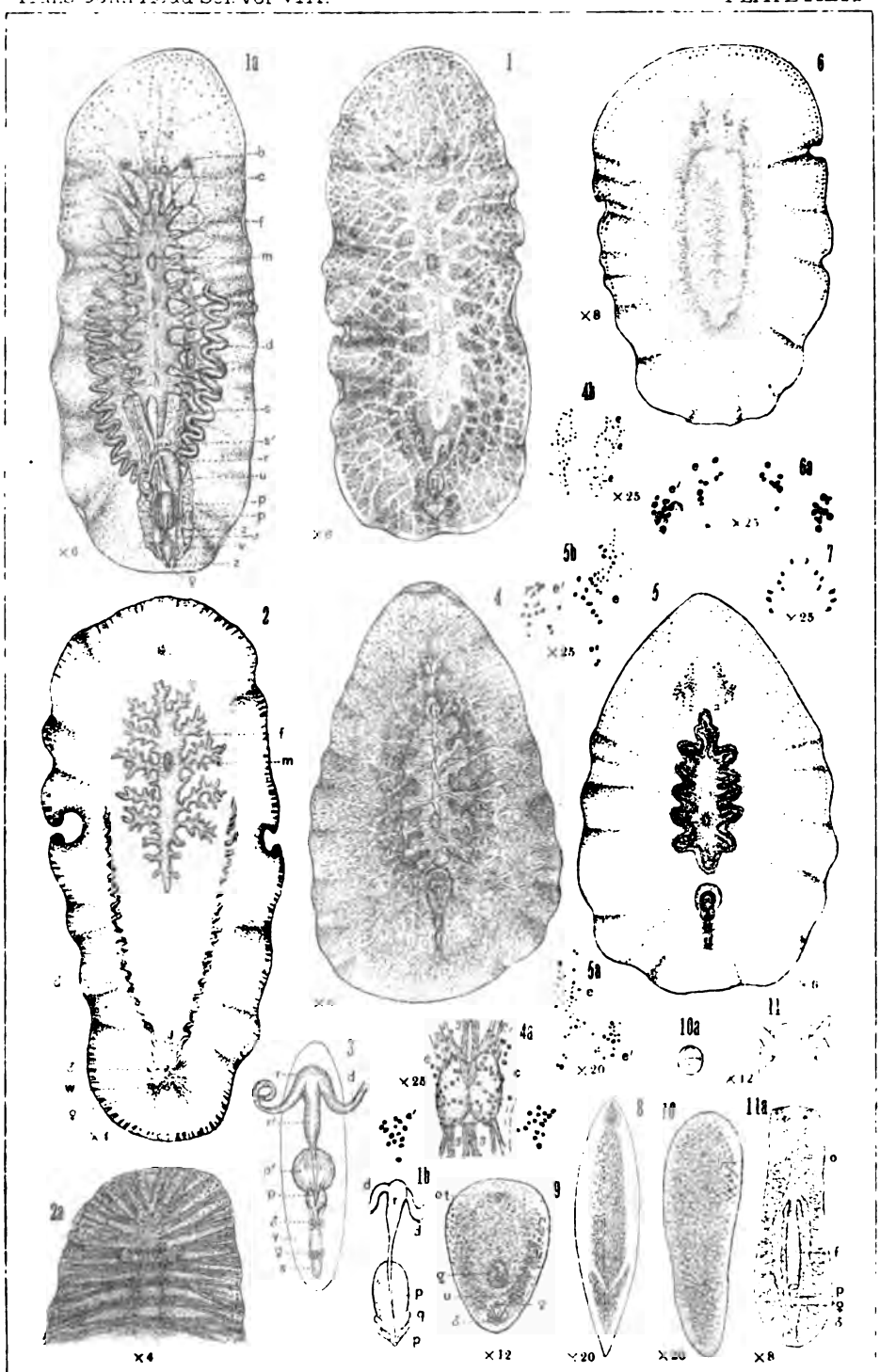
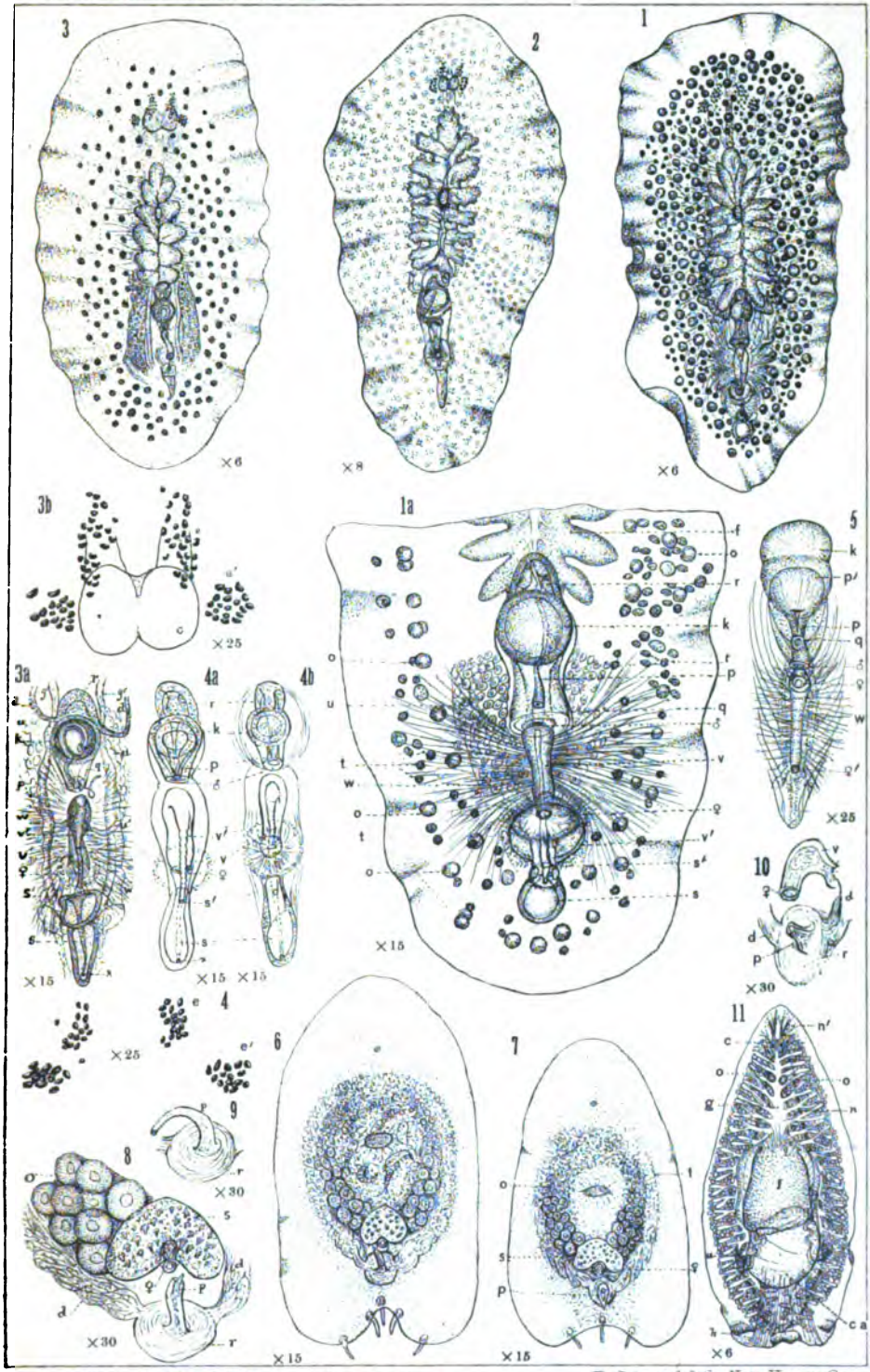



A E. Verrill and A H Verrill from nature

PLANARIANS




A. E. Verrill and A. H. Verrill from nature. E. Crisand, lith., New Haven, Ct.

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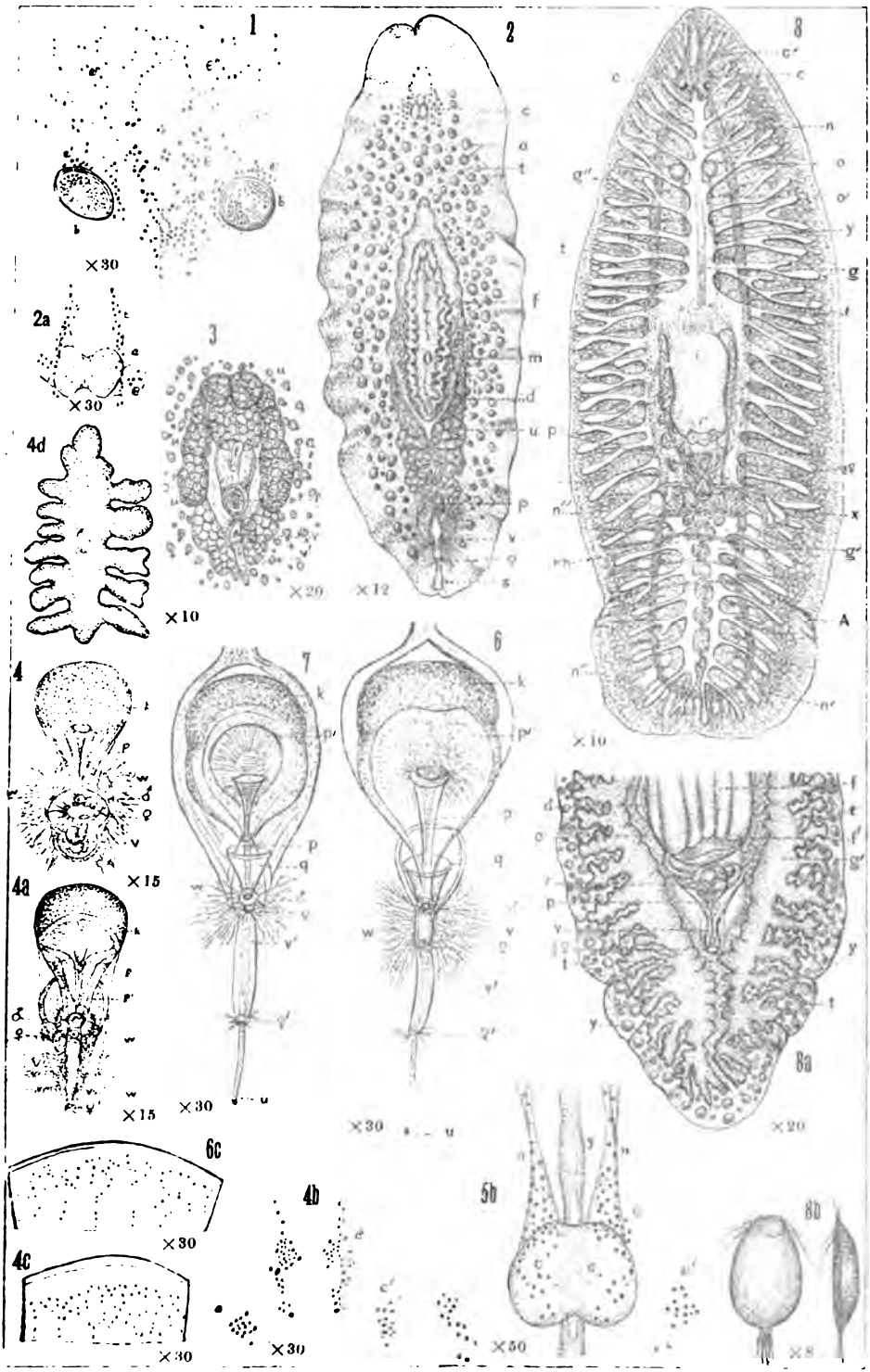


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